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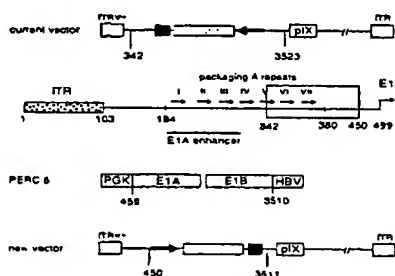
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovector backbone in the generation of the new vector.

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



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TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.
provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2
(serial number unassigned), filed September 15, 2000, March 27, 2001, and
September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first
generation adenovirus vaccines found to exhibit enhanced growth properties and
20 greater cellular-mediated immunity as compared to other replication-deficient vectors.
The invention also relates to the associated first generation adenoviral vectors
described herein, which, through the incorporation of additional 5' adenovirus
sequence, enhance large scale production efficiency of the recombinant, replication-
defective adenovirus described herein. Another aspect of the instant invention is the
25 surprising discovery that the intron A portion of the human cytomegalovirus (hCMV)
promoter constitutes a region of instability in adenoviral vector constructs. Removal
of this region from adenoviral expression constructs results in greatly improved vector
stability. Therefore, improved vectors expressing a transgene under the control of an
intron A-deleted CMV promoter constitute a further aspect of this invention. These
30 adenoviral vectors are useful for generating recombinant adenovirus vaccines against
human immunodeficiency virus (HIV). In particular, the first generation adenovirus
vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-
1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide
pharmaceutical products, and biologically active modifications thereof. Host
35 administration of the recombinant, replication-deficient adenovirus vaccines described
herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a
5 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA
10 binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0
5 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region
10 are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of
15 incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction
20 with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results
25 in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several
30 mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral
35 replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication-defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use
20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6[®] cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5 The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not
10 limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen
15 with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of
20 such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

 The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be
25 ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)
30 within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second
35 harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of
5 priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and
10 boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly
15 preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be
20 passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and
25 amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced
30 replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell
35 culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a 25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV 30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a 35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase
20 to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a
30 measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along
35 with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+bGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*III site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

10 "pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as
20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5 Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

 Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flagg-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flagg-SPA adenovectors constructed within
10 the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flagg-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1
15 antiparallel transgene orientation are represented.

 Figure 8A shows the experiment designed to test the effect of transgene orientation.

 Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20 Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

 Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

 Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6
25 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

 Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at
30 specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

 Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5
35 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5 Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed

10 herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

15 Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

20 Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

25 Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as
30 underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

35 Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with “*”, and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5 Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10 Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15 Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20 Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25 Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30 Figure 31 shows the intracellular γ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- γ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ γ IFN+ and CD4+ γ IFN+, respectively.

35 Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fusion frame.

5

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6[®] cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this
5 transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef
10 (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described
15 herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or
20 pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on
25 distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An
30 example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector
35 expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regimen in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a
5 nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost
5 administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at
10 least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine.
15 Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the
20 MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and
25 inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in
30 International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to
35 support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with

5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral
10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a
15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells
5 for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully
10 transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of
15 this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

20 Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient
25 to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed
30 *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin
35 resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors
5 not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol,
10 pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were
15 harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby
20 incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®],
25 from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

30 It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be
35 used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM $MgCl_2$; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM $MgCl_2$, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene
20 product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8
5 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early
15 (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of
20 the hCMV promoter and a 3' primer (designed to contain the *BglII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglII*. This fragment was then cloned back into the original
25 GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

30 The FLgag gene was excised from pV1JnsHIVgag using *BglII* digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *BglII* site. Colonies were screened using *SmaI* restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence
35 integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 ^a	10.8
PV1Jns-hCMV-FLgag-bGHpA ^b	16.6
pV1Jns-hCMV-FLgag-SPA ^{b,c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5 ^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

10

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above
 15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 μg and 200 μg .

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA ^a Promoter/terminator	Dose, ug ^b	Anti-p24 Titers (3 Wk PD1) ^c			SFC/10 ⁶ Cells (4 Wk PD1) ^d		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

^ain PBS^bl.m. Injections into both quads, 50 µL per quad^cn=10; GMT, geometric mean titer; SE, standard. error^dn=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector -"MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

(1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.

(2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.

(3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *Pac*I and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*I linearized pAdHVO (E3- adenovector) or *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I, *Bam*HI, *Xho* I, *Eco*RV, *Hind*III, *Sal* I, and *Bgl* II sites. This MCS was replaced with a new MCS containing *Not* I, *Cla* I, *Eco*RV and *Asc* I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *HindIII* (and *PacI* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *HindIII* (and *PacI* to remove the vector backbone) and then labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

5 Construction of the new shuttle vector containing modified gag transgene –
“MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHPA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeI1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeI1 shuttle vector.

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*I. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cl*aI overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bsf*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *PacI* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6[®] cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [³³P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *PacI/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11.

Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture.

5 Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

25 The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5 Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10 ⁶ cells/ml), Viability (%) Infection Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁵ vp/ml culture	Titer 10 ⁴ vp/cell	QPA 10 ⁵ TCID ₅₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.68, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)
P5	1.38, 93%	0.66, 47%	48	49	8.7	4.9	1.38	49	170
P6	1.04, 84%	0.68, 77%	47	48	5.8	5.6	1.42	41	200
P7	1.50, 84%	0.96, 81%	49.5	50	3.9	1.4	0.97	40	50
P7	1.09, 97%	0.76, 59%	50	52	5.2	4.7	1.70	31	170
P8	1.03, 94%	0.88, 64%	47.5	54	9.0	8.7	1.10	82	310
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	175
P10	1.09, 91%	1.08, 66%	47.5	58	3.0	2.8	1.16	28	100
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	62	210
P14	1.94, 92%	0.88, 67%	46	53	8.6	4.4			160
P15	0.97, 86%	0.64, 66%	47	47	6.9	7.1			250
									3.12 2.84 2.70 2.60 2.70 2.66 2.60 3.18 3.18 3.28 3.27 3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10 ⁶ cells/ml), Viability (%) Infection Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁵ vp/ml culture	Titer 10 ⁴ vp/cell	QPA 10 ⁵ TCID ₅₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 87%	1.28, 78%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)
P5	0.92, 89%	1.18, 77%	47	48	4.3	4.7	1.24	95	170
P6	1.55, 88%	1.26, 76%	49.5	50	1.2	0.8	0.56	21	30
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.16	34	130
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0.47	45	75
P9	1.20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25
P10	0.99, 82%	1.55, 86%	47	60	2.3	2.3	0.43	53	80
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110
P14	0.97, 86%	1.03, 98%	48.5	47	9.4	9.7			350
P15	0.87, 99%	0.97, 69%	49.5	49	5.3	6.1			218
									3.12 2.84 2.70 2.60 2.70 2.66 2.60 3.18 3.18 3.28 3.27 3.12 2.91 2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

MRKAd5gag(E3-)

	Xv (10 ⁶ cells/ml), Infection	Viability (%), Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 ⁶ vp/ml culture	Titer 10 ⁶ vp/cell	QPA 10 ⁶ TCID ₅₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 82%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.08, 87%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.84
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	2.86
P13	1.86, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.18
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.18
P15	0.87, 99%	0.84, 66%	49	49	4.8	5.5			196	3.28
										2.27
										3.12
										2.91
										2.78
										2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors ^a	$\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^e	0.42

^a $A_{260\text{nm}}$ absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

^d Research Ad5FLgag lot# 6399

^e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	^a MRKAd5gag	10 ⁷	25600	5877	4780
2	"	10 ⁹	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 ⁷	7352	2077	1620
4	"	10 ⁹	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 ⁷	12800	9905	236
6	"	10 ⁹	310419	99181	75165
7	^b mCMV FL-gag bGHpA [E3+] →	10 ⁷	44572	23504	15389
8	"	10 ⁹	941014	239068	190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10 ⁷	3676	934	745
10	"	10 ⁹	117627	17491	15227
11	research lot hCMV IntronA FL-gag bGHpA [E3-] <-	10 ⁶	528	262	175
12	"	10 ⁷	14703	5274	3882
13	"	10 ⁸	58813	14942	11915
14	"	10 ⁹	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10 ⁶	230	82	61
16	"	10 ⁷	4222	3405	1138
17	"	10 ⁸	19401	3939	3274
18	"	10 ⁹	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10⁶ dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10¹¹ vp and 10⁹ vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-
 10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assmumarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after
- 5 CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag ^a , 10 ¹¹ vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10 ⁹ vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^b , Clinical Lot, 10 ¹¹ vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10 ⁹ vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
^a MRKAd5gag (hCMV, bGHpA, E3+)								
^b original Ad5gag vector (hCMV/intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media ^a	Gag H ^b	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 ¹¹ vp	97N010	6	89	0	395	0	1058	0	1174	3	775	4	1074
		97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag 10 ⁹ vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot 10 ¹¹ vp	97X001	0	261	1	485	0	817	0	1220b	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 ⁹ vp	97N020	3	30	1	101	0	66	0	36	0	26	0	41
		97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Naïve	96R041	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	0	15	0	15	24	9

Based on either 4x10⁶ or 2x10⁶ cells per well (depending on spot density)

ND, not determined

^aMock or no peptide control

^bPool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10⁹ vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

15

EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

20

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after

5 review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-

10 type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly

15 (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It

20 is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol

25 protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease

30 (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

35 AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
 5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
 AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCTGACC
 10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
 CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCTCCC AAATCTACCC TGGCATCAAG
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGG ACCAAGGCCC TGACTGAGGT GATCCCCCTG
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGTGGC
 ATCAGGAAGG TGCTGTTCTT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
 CACTCCAAC TGGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
 30 TGCTCCCCCTG GCATCTGGCA GCTGGACTGC ACCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGA GTCCATGAAC
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCAT CCACAACCTT AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTTCAAGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:2) .

The present invention especially relates to an adenoviral vector vaccine which
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to
 deletion of the portion of the wild type sequence encoding the protease activity, a
 30 combination of active site residue mutations are introduced which are deleterious to
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein
 the construct is devoid of DNA sequences encoding any PR activity, as well as
 containing a mutation(s) which at least partially, and preferably substantially,
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCTTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
TGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGTGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTAAT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCTGCCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGTCTGGC
35 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCT CCACAACCTT AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 10 CAGATCACCA AGATCCAGAA CTTGAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
 NO:3).

15 In order to produce the IA-pol-based adenoviral vaccines of the present
 invention, inactivation of the enzymatic functions was achieved by replacing a total of
 nine active site residues from the enzyme subunits with alanine side-chains. As
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this
 IA Pol construct), with each residue being substituted for an Ala residue, respectively
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase
 function was abolished through three mutations at Asp626, Asp678 and Glu714.
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:4) .

As noted above, it will be understood that any combination of the mutations
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based
 adenoviral HIV vaccine of the present invention, either when administered alone or in
 a combined modality regime and/or a prime-boost regimen. For example, it may be
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,
 RNase-H, and integrase coding regions while still abolishing these enzymatic
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide
 such as is found in highly expressed mammalian proteins such as immunoglobulin
 leader peptides. Any functional leader peptide may be tested for efficacy. However,
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,
 preferably a leader peptide from human tPA. In other words, a codon optimized
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide
 at the amino terminal portion of the protein, which may effect cellular trafficking and
 hence, immunogenicity of the expressed protein within the host cell. As noted in
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

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25  GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
    CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
    GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
    CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
    CCCCAGAAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
30  GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
    GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
    GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCPTTAC
    CATCCCCTCC ATCAACAATG AGACCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
    GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
35  CAGGAAGCAG AACCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
    TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

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GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGTGGG
 TGTGCAGAAG ATCACCACCT AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 CCCTGAGTGG GAGTTTGTGA ACACCCCCCCT CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATT TTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGACAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)
 which comprises a leader peptide at the amino terminal portion of the protein, which
 may effect cellular trafficking and hence, immunogenicity of the expressed protein
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,
 any such leader peptide-based HIV-1 pol mutant construct may include but is not
 limited to a mutated DNA molecule which effectively alters the catalytic activity of
 the RT, RNase and/or IN region of the expressed protein, resulting in at least
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at
 least one point mutation which alters the active site and catalytic activity within the
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
 CTTGCTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
 CCCCAGAAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
 20 GGGGGATGCC TACTTCTCTG TGCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
 CATCCCCCTC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
 GGGCTGGAAG GGCTCCCTG CCATCTTCCA GTCTCCATG ACCAAGATCC TGGAGCCCTT
 CAGGAAGCAG AACCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCATT GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGGAGGC
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCAT TGTGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 GGAGGTGAAC ATTGTGACTG CCTCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 GAACCCCTG TGGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfrl
10 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCC GCCG CCGACAGGGT GAGGAGGACC GAGCCCCGCC
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
15 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCC GG CATC AGGTTC CCGG TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGCACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating
methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine
vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions
 have been elucidated, it has become clear that correct trafficking of Nef to the inner
 plasma membrane promotes viral replication by altering the host intracellular
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the
 20 infectivity of progeny viral particles. In one aspect of the invention regarding
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the
 adenovirus vector of the present invention is modified to contain a nucleotide
 sequence which encodes a heterologous leader peptide such that the amino terminal
 region of the expressed protein will contain the leader peptide. The diversity of
 25 function that typifies eukaryotic cells depends upon the structural differentiation of
 their membrane boundaries. To generate and maintain these structures, proteins must
 be transported from their site of synthesis in the endoplasmic reticulum to
 predetermined destinations throughout the cell. This requires that the trafficking
 proteins display sorting signals that are recognized by the molecular machinery
 30 responsible for route selection located at the access points to the main trafficking
 pathways. Sorting decisions for most proteins need to be made only once as they
 traverse their biosynthetic pathways since their final destination, the cellular location
 at which they perform their function, becomes their permanent residence.
 Maintenance of intracellular integrity depends in part on the selective sorting and
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-
5 alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector
10 or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef
15 protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1
20 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down
25 regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector
30 HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter
35 function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

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CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGCGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG
GCCCAGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCATGTG
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTTC ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfrl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
 TGTCCTACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
 ACACCCCCGG CCCCAGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCACCC
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGACT
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue
 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GCGCCGTGG ACCTGTCCCA
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG
 GCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCC ACCCATGTC
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT
 GGCCTTCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,
 regardless of codon usage, which expresses a wild type or modified Nef protein as
 35 described herein, including but not limited to modified Nef proteins which comprise a
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*I site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using
 5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its
 10 isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)ClaI. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA
 15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-
 25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing
 30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in

Example 19 above, the vector

MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to

5 contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

15 MRKpdeIE1+CMVmin+BGHPA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHPA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHPA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHPA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

35 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at $\leq -60^{\circ}\text{C}$. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

10 The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent
15 the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR
20 product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4
25 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel
30 orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length
35 IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

EXAMPLE 22

5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial
30 homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁷ vp and 10⁹ vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were
 5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following
 10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were
 15 collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁹ vp and 10¹¹ vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either
 20 10⁹ vp and 10¹¹ vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0)
 25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μ L of 1 μ g /mL HIV-1 RT protein
 30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 μ L of 1 μ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200 μ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was
 35 performed followed by 4-fold serial dilution. 100- μ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO₄ per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELISpot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF γ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10⁶/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of either 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 μ g/mL mouse anti-human IFN- γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μ L of cell samples (4-5x10⁵ cells per well) and 50 μ L of the antigen solution were added. To the control well, 50 μ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 μ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁷ vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 ⁷ vp	2 1	310419 919	301785 372	163020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10 ⁹ vp	2 1	1638400 ^b 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2083(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10 ⁷ vp	2 1	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 ⁹ vp	2 1	1638400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNear or at the upper limit of the serial dilution; hence, could be greater than this value^cNo. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. The immune response were analyzed using similar protocols and the results - listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELISpot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers ^a			SFC/10 ⁶ cells ^b		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 ⁷ vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10 ⁹ vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10 ⁷ vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 ⁹ vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10 ⁷ vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10 ⁹ vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	26(3)

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNo. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monkey #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+) 10^{11} vp	99C100	1	0	0	1	38	31	0	52	148	0	49	715
	99C215	1	2	2	10	88	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+) 10^9 vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	182	4	36	156	5	38	108
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-) 10^{11} vp	99D239	5	2	2	20	82	172	1	66	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	236	1	24	264
MRKAd5hCMV-IAPol(E3-) 10^9 vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Ndve	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined

Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), 10^{11} vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), 10^{11} vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), 10^9 vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef

- 5 constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ¹¹ vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁹ vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ¹¹ vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁹ vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

- 15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef
Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 ⁶ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 ⁶ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
- 20 MRKAd5gag. PER.C6[®] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
- 25 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6[®] cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 ⁶ cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁴ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef (MRKAd5nef)	Pool	1.11, 91%		60	1.5	1.4	50	2.8
	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

EXAMPLE 27

Characterization and Large Scale Production of

MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6[®] cells at a concentration of 0.2x10⁶ cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10⁶ cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10^7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10^7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$ -biased or $CD8^+$ -biased, and (b) boosting with the MRKAd5gag
30 construct produced in all cases a strongly $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

Grp#	Priming T=0, 4, 8 wks DNA/5 mgs PBS (D101)	Boost T=28 wks MRKAd5gag(E3+) 10 ⁷ vp	Munk#	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1				NA	NA	3	35	15	71	4	224	8	115	6	05	19	556	0	316
				0	0	0	15	0	46	0	68	0	76	0	35	3	1705	1	755
				5	11	0	35	3	51	3	46	2	89	8	65	10	989	0	355
2				0	4	1	60	0	111	5	270	4	260	8	232	3	959	19	1345
				4	0	1	101	0	254	0	781	5	452	0	321	0	1815	1	1059
				9	8	1	10	4	71	4	164	8	104	5	85	11	836	6	241
				NA	NA	0	31	0	288	0	530	19	374	9	251	8	1549	20	1734
				9	12	4	36	1	119	0	439	0	425	0	316	4	1229	5	1354
3				10	4	1	59	5	264	19	425	6	105	9	205	18	585	8	404
				1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
				8	6	0	6	3	119	0	274	6	282	1	208	0	836	1	828
				4	3	0	26	1	91	0	139	0	164	1	82	5	543	1	349
				1	0	0	136	0	316	1	609	5	625	1	759	0	2278	4	1831
4	none	None	98D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

5 The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IAPol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IAPol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IAPol fusion gene.

EXAMPLE 30

20 Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein
30 sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

5 **Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 ¹⁰ vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 ⁸ vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 ¹⁰ vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 ⁸ vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 ¹⁰ vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 ⁸ vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ¹⁰ vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ⁸ vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 ¹⁰ vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 ⁸ vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10⁶ PBMC.

WHAT IS CLAIMED IS

1. A recombinant adenoviral vaccine vector at least partially deleted in
5 E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from
about base pair 1 to between from about base pair 400 to about
base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region
corresponding to from about base pair 1 to about base pair 450 of a wildtype
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides
15 corresponding to between from about base pair 3511 to about 3524 to about base pair
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;
b) a heterologous promoter operatively linked to the nucleic acid
encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6[®] cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell
15 line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene
20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6[®] cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs
15 corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of
SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and
20 SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10 75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;
and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with
claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to a single promoter; and the encoding nucleic acid sequences
operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

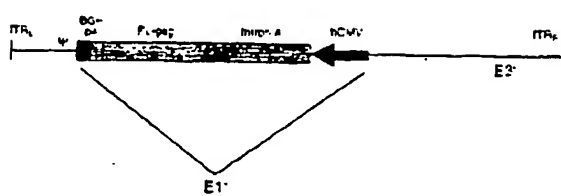


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctgggtggtagctggacaagtgggagaagatcaggctgaggcctgggtg
caagaagaagtacaagctaaagcacatgtgtggccctccagggagctggagaggtttgctgtgaacctggc
ctgtggagacctctgaggggtgcaggcagatcctggccagctccagccctccctgcaaacaggctctgagg
agctgaggtccctgtacaacacagtggctacccgtactgtgtgcaccagaagattgatgtgaaggacaccaag
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgtgtgtggc
acaggcaactccagccagggtgtccagaactacccatgtgtgcagaacctccagggccagatgtgtgcaccag
ggcatctccccccggaccttgaatgacctgggtgaagggtggtaggagaaggccttctccctgagggtgatccc
catgttctgtccctgtctgaggggtgtccacccccaggacctgaacaccatgtgtgaacacagtggggggccatc
aggctgccatgcagatgtgtgaaggagacatcaatgaggaggctgtgtgtgtggacaggctgtcatcctgtgc
acgtgtggcccatgtgtcccccggccagatgaggggagcccaggggctgtgacatgtgtggcaccacctccacct
ccaggagcagattggctggatgaccaacaaccccccatcctgtgtgggggaaatctacaagaggtggatcat
ccttggccctgaacaagattgtgaggatgtactccccaccctccatccttggacatcaggcaggggccccaaggag
cccttcagggaactatgtggacaggttctacaagaccttgagggtgtgagcaggcctccaggagggtgaagaact
ggatgacagagacctgtgtgtgcagaatgccaaacctgactgcaagaccatcctgaaggcccttggggcctg
ctgccaccttggaggagatgtgacagcctgtccaggggtgtggggggccctgtgtcaaggccaggggtgtgt
gtgaggccatgtccagggtgaccaactccgccacatcatgtgtgagagggggcaacttcagggaaccagag
gaagacagtgaagtgtctcaactgtgtgcaagggtgggccacattgccagaactgttagggccccaggaaga
agggtgtgtggaagtgtgtgcaaggaggggccaccagatgaaggactgcaatgagaggcaggccaacttctg
ggcaaaatctggccctcccaagaaggcaggcctgtgcaacttctccagtcaggcctgagcccacagcccct
cccgaggagtccttcagggttggggaggagaagaccacccccagccagaagcaggagcccatgtacaagg
agctgtacccccgtggcctccctgagggtccctgtttggcaacgaccttctccaglaaaataaagccgggca
gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

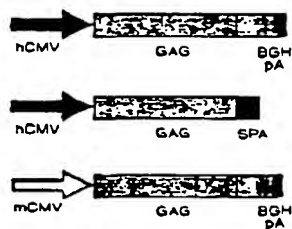


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

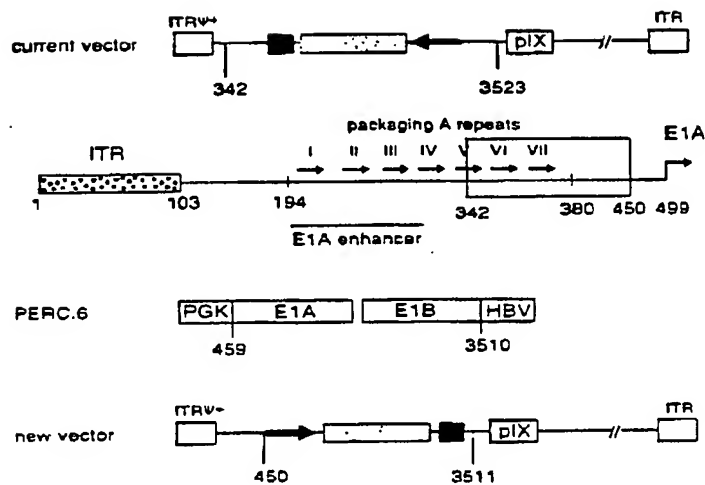


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

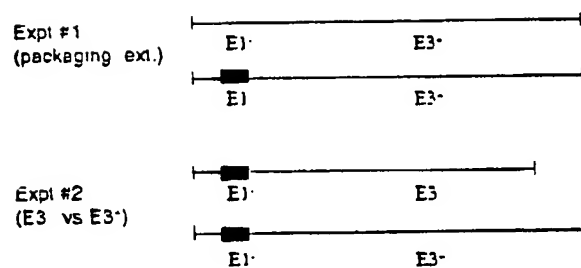


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

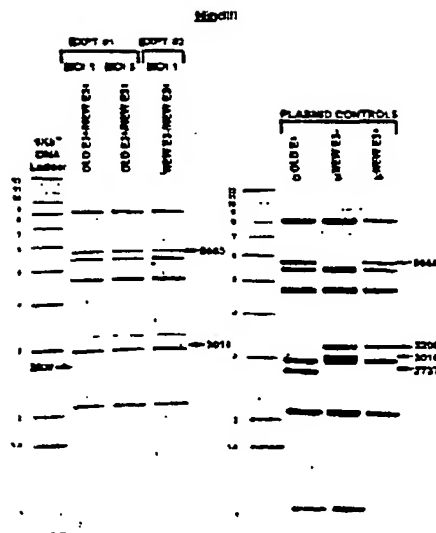


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

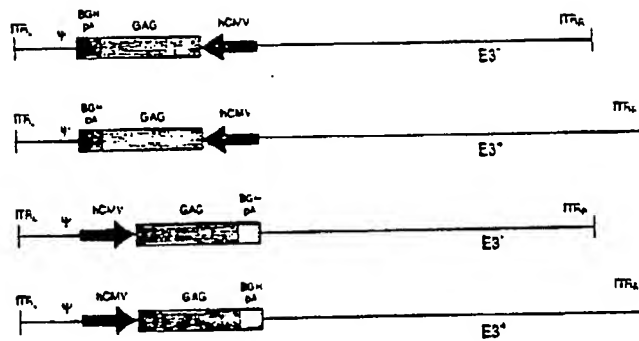


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

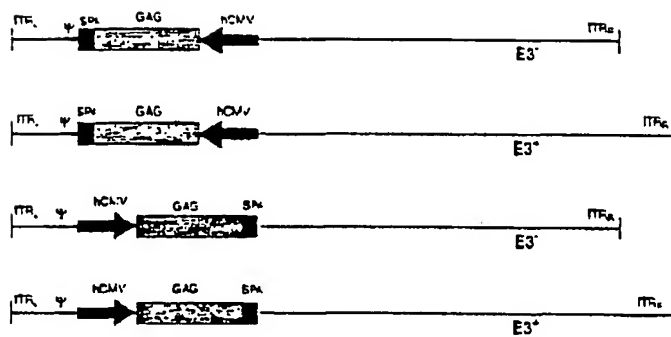


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

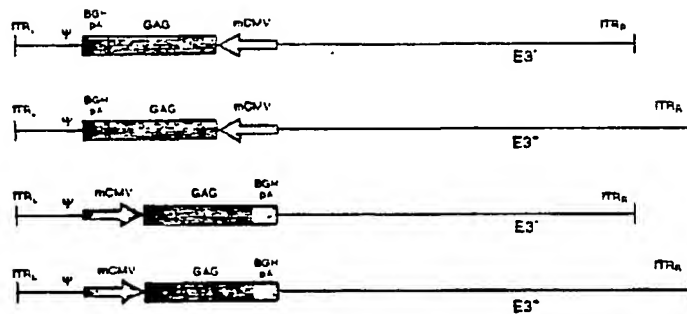


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3⁻ and E3⁺ backbones were constructed.

Plasmid mixing expt: (orientation)

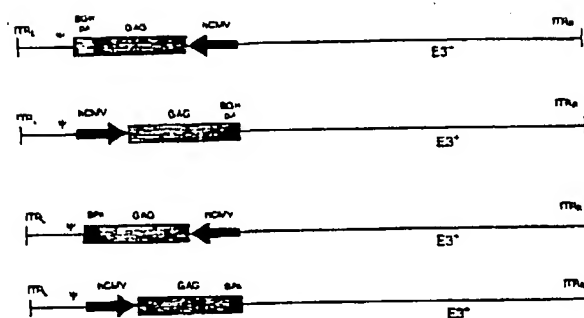


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

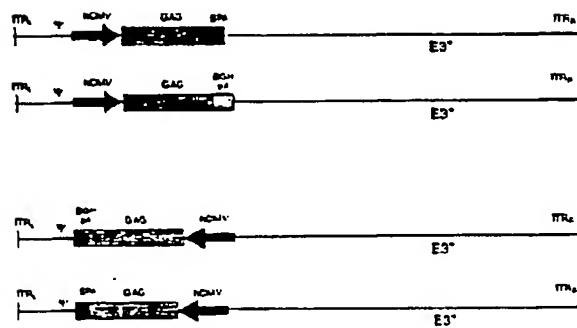


Figure 8B: Effect of polyadenylation signal

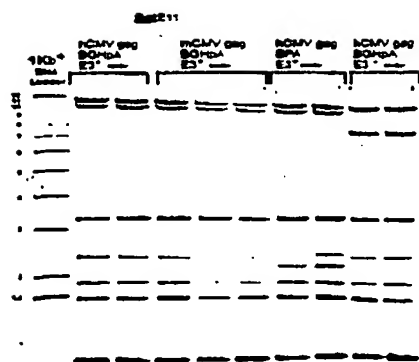


Figure 9: Viral DNA from the four Adgag candidates at P5, following *EstE11* digestion.



Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).



Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

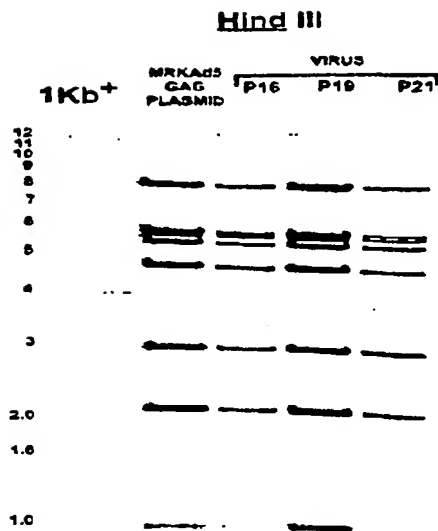
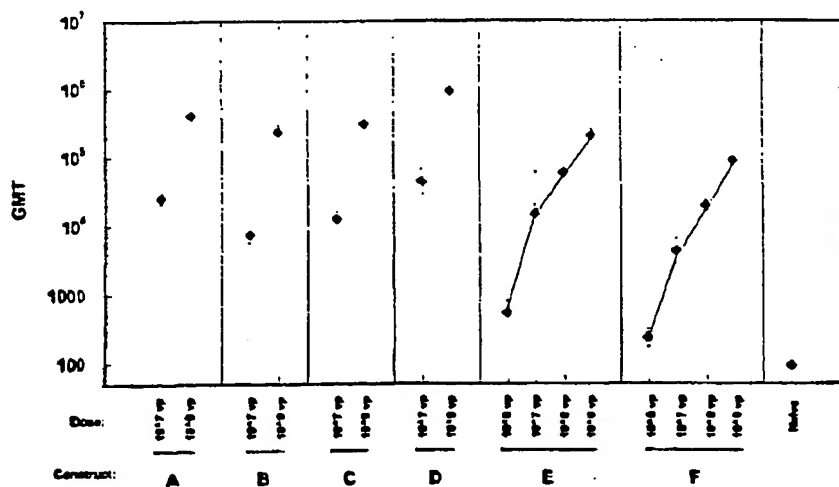


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13
Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁺ hCMV-FLgag-bGHpA; (C) MRKAd5 E3⁺ hCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (E) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



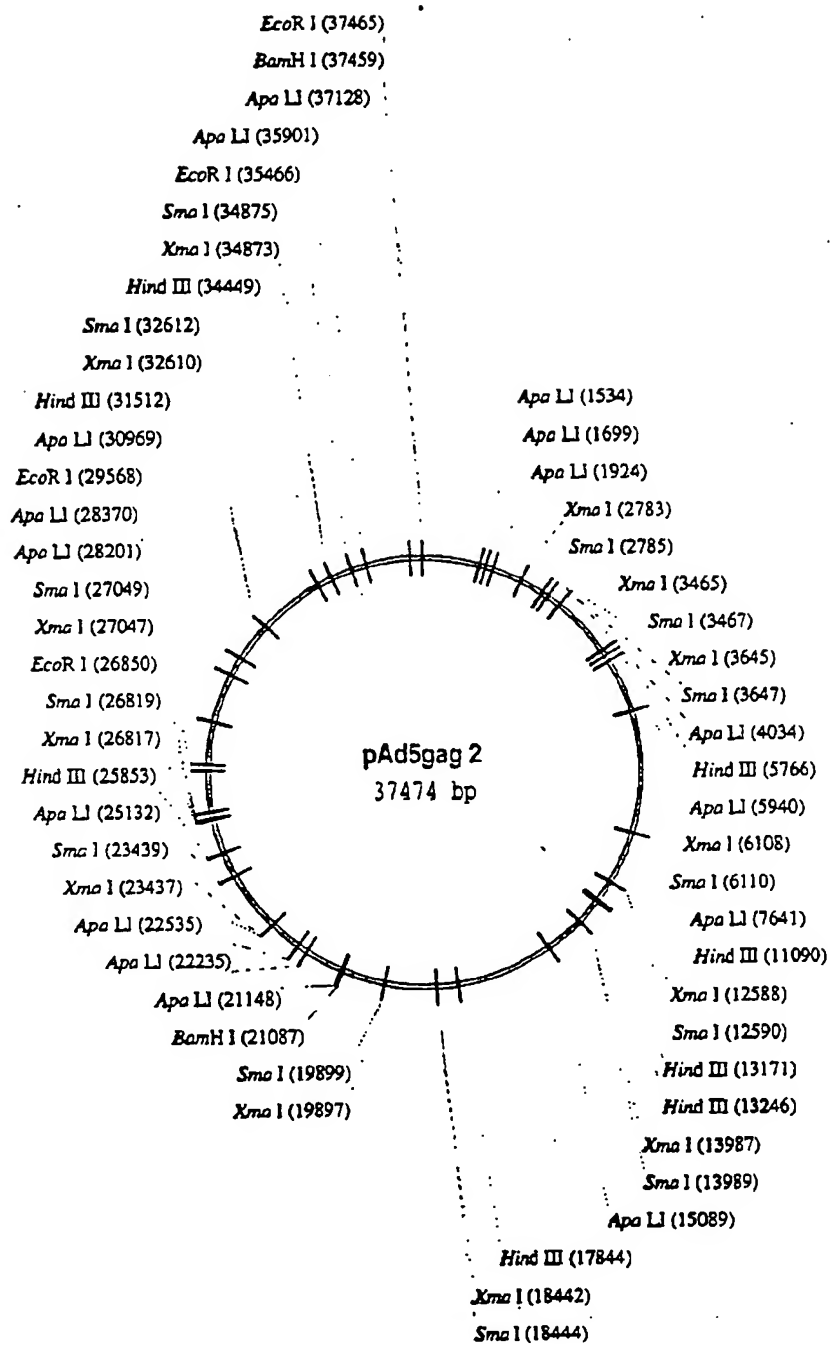


Figure 14

[illegible]

Figure 15A

pHRKAI'qan MER62

1701	CACAGAGCCA	TCCTCCCTCC	GACCTCTAAT	GCCCTCTTGA	AGCTCTTCTA	AGAGAGGC	TTCTCCCTG	AGTGTATCCC	CATCTCTCT	GCCTCTCTG
	GTCTCTGGT	AGAGGGGAC	CTGGTACTTA	CGGCTTACT	TTCTCTCTA	CTCTCTCTG	AGAGGGGAC	TCCACTAGG	GTACAAGAG	CTGTACAGAG
1801	AGGTGACAC	CCCCAGGAC	CTGACACTA	TTCTGAATC	ATCTGAATC	ATCTGAATC	CTCTCTCTG	CTCTCTCTG	AGTGTATCCC	CTGTACAGAG
	TCCTCCCTG	GGGGTCTG	GACTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG
1901	TGAGTGGAC	AGGTCTGAT	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG
	ACTCACCTG	TCCAGCTAG	GACAGCTAG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG
2001	CAGGAGCAGA	TTGCTCTGAT	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG
	GTCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG	AGCTCTCTG
2101	ACTCTCTCTG	CTCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG	GACCTCTCTG
	TGAGGGGCTG	GAGGTAGGAC	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG
2201	CCAGAGGCTG	AGAACTCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2301	GAGGAGTGA	TGAGAGGCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2401	TGAGAGGCTG	AGAACTCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG	TGAGAGGCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2501	GAGGAGTGA	TGAGAGGCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG	CTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2601	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2701	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2801	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
2901	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
3001	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
3101	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
3201	AGGAGTGA	ACTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG
	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG	GTCTCTCTG

Figure 15B

pMRKAd5-qag MEH682

3301	TTGAGGACG CAGCTTCGG CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	TTGAGGACG CAGCTTCGG CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	TTGAGGACG CAGCTTCGG CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
3401	AACCTCTGC GTGCGAGCG GCGGCGAAG CTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	AACCTCTGC GTGCGAGCG GCGGCGAAG CTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	AACCTCTGC GTGCGAGCG GCGGCGAAG CTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
3501	GAGGCGAAG TAGCGCGCG CTTACTCTTA ACTGCTGAG AAGCGCTTC TTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG	PsII	GAGGCGAAG TAGCGCGCG CTTACTCTTA ACTGCTGAG AAGCGCTTC TTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG	GAGGCGAAG TAGCGCGCG CTTACTCTTA ACTGCTGAG AAGCGCTTC TTTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG
3601	TCGCGCGAG CAGGCTTTC CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	TCGCGCGAG CAGGCTTTC CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	TCGCGCGAG CAGGCTTTC CCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
3701	AGACCGGTC GTCCAGAGC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	AGACCGGTC GTCCAGAGC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	AGACCGGTC GTCCAGAGC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
3801	GTGCTCTCT GTCTTTATTT AGGCTTTC GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	GTGCTCTCT GTCTTTATTT AGGCTTTC GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	GTGCTCTCT GTCTTTATTT AGGCTTTC GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
3901	CACAGACGA CAGAAATATA TCCCCAAAC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA	PsII	CACAGACGA CAGAAATATA TCCCCAAAC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA	CACAGACGA CAGAAATATA TCCCCAAAC GCGCTCTTA GCTGCTGAG CATTCTGAG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA
4001	AAAGGTGACT CTGGAATGTC AGATACATG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	AAAGGTGACT CTGGAATGTC AGATACATG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	AAAGGTGACT CTGGAATGTC AGATACATG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4101	TTTCCACTGA GACCTACAG TCTATGTAC CGTATTCGG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	TTTCCACTGA GACCTACAG TCTATGTAC CGTATTCGG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	TTTCCACTGA GACCTACAG TCTATGTAC CGTATTCGG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4201	GATCCAGTCG TAGCAGGAG CTTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	GATCCAGTCG TAGCAGGAG CTTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	GATCCAGTCG TAGCAGGAG CTTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4301	CTAGGTCAGC ATCGTCTCG CCGCTCTTA GCTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	CTAGGTCAGC ATCGTCTCG CCGCTCTTA GCTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	CTAGGTCAGC ATCGTCTCG CCGCTCTTA GCTGCTGAG CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4401	CGTTAAAGCT CGATGCGTG CATAGCTCG GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	CGTTAAAGCT CGATGCGTG CATAGCTCG GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	CGTTAAAGCT CGATGCGTG CATAGCTCG GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4501	GCCAAATCCA CCGTACCCAC GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	PsII	GCCAAATCCA CCGTACCCAC GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG	GCCAAATCCA CCGTACCCAC GTATGACCC CATTACATG CTTACTTTC AATTACTTC CTTCTCTAG CCGCTTCCA AACACTGCG
4601	TTGCTGACG TTTCTGACG GAGCAAGCT TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG	PsII	TTGCTGACG TTTCTGACG GAGCAAGCT TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG	TTGCTGACG TTTCTGACG GAGCAAGCT TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG
4701	CTCTATCTGC AAGACGCTTC CTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG	PsII	CTCTATCTGC AAGACGCTTC CTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG	CTCTATCTGC AAGACGCTTC CTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG TTTCTGACG
4801	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG	PsII	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG
4901	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG	PsII	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG	CCACAGCTCG GTGCTGACG CAGTGGACGA GATGCGTAG AGCTAGTCC TATAGCTCG TATAGCTCG TATAGCTCG TATAGCTCG

Figure 15c

4901	GGTACGGCTT	AGGGCTGTCC	TCTGTGTGCT	GATGGCTTCC	CGGCTTCCG	CTCTGCTGTC	GGCTAGCTTAC	CAATTGACCA	TGGTGTCTATA	GTCCACATCC
5001	CCACGGCAAC	TCCGACCAAG	ACGACCAAGA	CTTCCGTACG	GGCTAGACGG	GTACTGCTAC	GGCTAGCTTAC	GTAACTGCT	ACCACAGTAT	CAGCTGCGGT
	TCCGCGCGCT	GGCGCTTTCC	GGCCAGCTTG	GGCTTTTAT	ATGCTTCCA	CTATGCTTAC	TGACAGCTTT	TGAGCGCTA	GAGCTTGGCC	GGCTTAAATA
	AGCGCGCCGA	CCGGGACCG	CGGTGTGAAC	GGAACTTCC	TCCGCTGCT	GGCTGCTGCT	AGCTCTTAAA	ACTCCCGCAT	CTCCCGCAT	GGCTTTTAT
5101	CGATTTCGG	GGATTAAGCA	TCCGCGCGCC	AGGCTCTTCA	GATGCTTCTG	CAATTCTAGCA	GTCAATCTAG	CTCTGGCGCT	TGCGGGTCA	AAATCAGCT
	GGCTAAGGCC	CGTCAATCGT	AGCGCGCGCG	TCCGGGGCGT	CTTCCAGAGC	CTTCCAGAGC	CTTCCAGAGC	GAGACCGGCA	AGCGCGCAT	TTTGTGCTAA
5201	TCCCGCANCC	TTTTTGTATC	GTCTTATACC	TCTGTCTTCC	ATGAGCTGCT	GTCCAGCTCT	GGTAAAGAAA	AGGCTGTCCG	TGTTCCCGTA	TACAACTT
	AGCGGGTACG	AAATCACTAG	CAATAGATCG	AGACCAATCG	TACTCTGCTA	CAGTGGCAG	CGACTGCTTT	TCCGACAGCC	ACAGGGCAT	ATGCTCTGAA
5301	AGAGGGCTGT	CTTCTGAGCG	ACAGGGCTCC	TCTTCTCTCT	ATGCAACTC	GTACCACCT	GACAGAAAG	CTCGGTCTCA	GGCGAGCAG	AGAGAGCTAT
5401	TCTCCGAGCA	GGAGCTCGCC	ACAGGGCTCC	AGGAGAGCA	TATCTTTTAC	CTTGTGTGAG	CTCTGTCTTC	GAGCGAGCT	CGGTGTGTC	TTCTCTCGAT
5501	AGTGGGAGCG	GTAGCGCTCG	TTTCTCACTA	GGGGTCTCAC	TCTCTCCAG	CTCTGTGAG	ACATCTGCC	CTCTTCTGCA	TCAAGGAGG	TGATTTGTCT
	TCACTCTTCC	CATCTCCAGC	ACAGGTGAT	CGCCAGGTG	CTCTGAGTCC	CAGACTCTG	TGTACAGCG	GAGAGCGCT	AGTTCTTCC	ACTAACCA
5601	GTAGGTATAG	GGCATCTGAC	CGGTGTTCG	TGCAAGGTG	CTTAAATAG	GGGTGAGCC	CGGTGTGTC	TCACTCTCT	CGCATCTCT	GTCTCGAGG
	CATCCAGATC	CGGTGCACTG	GGCCAGACAG	ACTTCTCTCC	CAATTTTCT	CGCACCTCG	CGCAAGCAG	AGTCAGAGAA	GGCTTAGCGA	CAGACCTTCC
	CGCACTCTCT	GGGTGTGAGTA	CTCTCTCTGA	AAAGCGGCA	TGACTCTGTC	GTATAGATTC	TCACTTTCTA	AAACAGGAG	GGATTTGATA	TTTCACTCTG
	CGGTGTGAGAA	CGCCACTCAT	GAGGTAGACT	TTTCTGCTCT	ACTGAAGAG	CGATCTTAC	AGTCAAAAG	TTTTTCTCT	CTTAACTAT	AGTGTAGCT
5701	CGCGGGTAT	GGCTTTAGCG	GTCCCGCAT	CGATCTGCTC	AGAAAGAGCA	ATCTTTTTCT	TGTCAACTT	GGCTGAAAC	GAACGGTAGA	GGTCTTTGTA
	GGCGCCACTA	CGGAACCTCC	CACCGGGTA	GGTAGACCAG	TCTTTTCTCT	TAGAAAGCA	ACAGTTGCA	CGACGGTTG	CTGCGCATCT	CGCCCAACT
5801	CAGCACTTGG	CGATGAGCG	CGAGGGTTTG	GTTTTGTCTG	CGATCTGCTC	GGTCTGTGTC	CGTATGCTT	AGCTGCACT	ATTCGCGGC	AACTGACCTC
	GTCTGTGAAAC	CGCTTACTCG	CGTCCCAAC	CAAAACAGC	GTAGCGCGCG	CGAGGAACCG	GGCTTACAAA	TGCAAGTCA	TAAAGCGCGG	TTCTGCTGCG
5901	CATTGCGGAA	AGACGGTGGT	GGCTGTGTCG	GGCAACAGCT	CGACGCTCCA	ATCTCGGTTG	TGCAAGGTGA	CAGGTTCAAC	CGGTGTGCT	AGCTCTCTCT
	GTAAAGCGCTT	TCTGGCAGCA	CGCGAGCAGC	CGCTGTGCTCA	CGTGTGCTG	TGCGGCAAC	AGCTTCCACT	GTCTCAGTTG	CGTCAACCGA	TGCAAGCT
6001	GTAGCGGCTC	GTCTGTCTCG	CGCGAGCGGC	CGCGCTCTG	CGCGAGAGT	GGGTGTAGG	GGTCTAGCT	GGGGGTCTG	GGCGGAGAC	CGTCTCACT
	CATCTCCGAG	CMACAGGTC	GTCTCTCGCG	CGCGGAATCC	CGCTCTCTTA	CGCTCAATCC	CGCAATGAG	GGAGAGCAG	CGCCCGAGAC	GGATGTCTCA
6101	AAAGCCCGCG	CGCAAGAGC	GTCTCTCTATC	GTAGTCTATC	TTTCAATCTT	GGAGTCTTAG	GGCTCTGTC	CATCGCGGG	CGGCAAGGC	GGCTCTGAT
	TTTCTGCGGC	CGTGTGCTCG	CGCGAGCTTT	CATCAGATAG	ACGTAGGAA	CGTTGAGATC	GTGAGAGAG	GTACCGGCG	GGCTTTCCG	CGCGAGGATA
6201	GGTTTGAAGT	GGGAGACCCA	TGGATGTAGG	TGGTTTAGCG	CGAGTCTGTA	CATGCGGCA	ATGCTGTAAA	GGTAGAGGG	CTCTCTTGAT	TAATCCAAAT
	CGCAACTCAC	CGCTTGCGGT	ACCTTACCGC	ACCGACTGTC	GGCTCTCGCAT	GTACGGCGTT	TACAGAGTT	GCATCTGCC	CGAGAGCTCA	TTAGGTTCTA
6301	ATGTAGGGTA	CGATCTTCCA	CGCGGTATATC	TGGCGGCTAC	GTAATCTGAT	ATTTCTGCTA	AGGAGAGG	CAGCTTGGA	GGGTCTGGA	TAGCGGGCTG
	TATCATCTCAT	CGTAGAAGTT	TGCGGCTTACG	ACCGCGCTAC	GTATCTGCTA	CTCAAGCAGC	TCCCTCTGCT	CTTCAAGCGCT	GGGTCTAAG	ATGCCCGCT
6401	CTGCTCTGCT	CGTAGAAGTT	TCTGCGCTAC	GATGCGCATG	GATCTTGTAG	ATATGCTTTC	ACGCTTAAAG	AGTTGTAGC	TGCGTCTGCT	GAGACTTACT
	GACAGAGAGCA	GGCTTCTACT	AGACGGACT	CTACCGCTACA	CTCAACTTAC	TATACCAAC	TGGGACTTC	TGCAACTTCC	ACCGAGACA	CTCTCTGATG

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9701	ACAAAGCGGT GGTATGCGCC CGTGTGATG GTGATAGTC AGTTCGAT ATAGCTGCT GTGACCGCG GTGACGAGC CTGCTGATC	TCGCTGATC
	TGTTTGGCCA CCAATGCGG GCACAATAC CAAATTCAG TCAAGCTATA TTAGCTGCTC AATGCGCA CCACTGCGC GACGCTCG AGCCATAT	
9801	TGAGAGCGGA GTAGGCGTC GAGTCAAATA GTTATGCTT GAACTCTTC ATCAATGCT GGTATGCGC CAAAGATGC GCGCTAGAC	GGGCTAGAC
	ACTCTGCGCT CATTCGGAG CTCAGTTAT GCATCAATA GCATCAATA GCTTCAAGG TGTTCATCA CCAATAGCTG GTTTCACG CCGCGCGA CCGCATCTC	
9901	GGGCGAGGT AGGATGCGG GGGCTGCTG GAGTCAATG GATGATGCT TCAATATA GATGATGCT GATGATGCT TCAATGAT TCAATGAT	GGGCGAGC
	CCGCGTCCA TCCGACCGC CCGGAGCGC CCGTCTGAG AGTTGTAT CCGTACTAT AGCATCTAC ATGAGCTGT AGTTCATTA CCGCGCGC	
10001	GTGCTGAGG CCGCGGAA GTGCGAGG CCGTCTGAG TGTGCGAG CCGTCTGAG TGTGCGAG CCGTCTGAG TGTGCGAG TGTGCGAG	ATGCTGAGC
	CACGACCTC CCGCGCTT CAGCGCTTC GCGAGCTC GCGAGCTC GCGAGCTC GCGAGCTC GCGAGCTC GCGAGCTC GCGAGCTC	
10101	AATGCTGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC GCTGAGAC	GGGCGAGC
	TGAGACTG CGAGATCG CAGATCTG CAGATCTG CAGATCTG CAGATCTG CAGATCTG CAGATCTG CAGATCTG CAGATCTG	
10201	GGTTCGAG CCGTATCG CCGTATCG CCGTATCG CCGTATCG CCGTATCG CCGTATCG CCGTATCG CCGTATCG CCGTATCG	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10301	TGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	AACCGGAG GAGTCTG CAGTCTG CAGTCTG CAGTCTG CAGTCTG CAGTCTG CAGTCTG CAGTCTG CAGTCTG	
10401	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10501	TGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10601	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10701	TGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10801	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
10901	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
11001	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
11101	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	
11201	GTGCTGCTC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC TCGAGCGC	GGGCGAGC
	CCGAGCTG GCGATAGG CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC CCGAGCGC	

Figure 15g

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11301 TCGATTTCAT AMCATCTCG CAGAGATATAG TGTATATACGA TGTATATATTC ATCTATATATG AGCATATATG CCGCATCAAC TATTCCATGC TTAGCTCTGG
 11401 AACTAATATG TTTGTATGAC TATATATATC TGTATATATCT CTTATATATAC TGTATATATG TGTATATATG TGTATATATG TGTATATATG TGTATATATG
 11501 CTTCAAAATG CCGCGTTCT ATATATATATG GGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 11601 ACCATTGACG AGCATATATG CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 11701 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 11801 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 11901 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12001 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12101 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12201 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12301 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12401 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12501 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12601 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12701 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG
 12801 CCGCATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG TGTATATATATG

Figure 15H

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12901 GGCATGATATG CCTCAACAG GCGCTTTATC AACTGCTTAA TTATG¹ ACTT GATATGATATG GATGCTGCTTA ACCGCTGATTA TTTCACCAAT GCTATCTTGA
CCGTATACATAC GGAGTTTGGC CCGCAATATAG TTATGATATG AATG² TAAA CTTATGCTATC GATGCTGCTTA TTATGCTATC TATGCTATC TATGCTATC
13001 ACCGCTGCTG GCTACGCTG CCGCTTTTCT ACACGCTGCTT ATTCGCTATG CTTATGCTATC AATGCTATG CTTATGCTATC TATGCTATC TATGCTATC
TGGGCTGATC CGATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13101 TTGCTGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13201 CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13301 AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13401 GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13501 GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13601 AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13701 TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13801 GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
13901 AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
14001 GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
14101 AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
14201 GATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
14301 AAGGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
CTATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
14401 TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG
TATGCTGCTG GATCAATAG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG TATGCTGCTG

Figure 151

[illegible]

Figure 15 J

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16101	CCAGGTGATC GCGCGGAGA TCTATGTC CCGGAGAGAG GAGAGACAGG ATTATAGCT CTGAGAGCTA AGCGGGTCA AAAAGAAAA GMAAGATCAT GGTCCAGTAG CCGCGCTCT AGATACCGG GGGCTCTTC CTATGCTCC TAATGTTCTG GACTTTGAT TTGCGCCAGT TTTTCTTTT CTTTCTACT
16201	GATGATGAG TTGAGGACGA GGTGAAATG GTTACGCTA GTTATGCTAG GTGAGTGTTA CATGTGMAAG GTGCGCGCT AAAAGCTGTT TTGCGACCC CTACTACTTG AACTGTCTCT CCACCTTGAC GACTGTGAT GTGCTGCTG CCGTTCCTAT GTACCTTTTC CAGCTGCGCA TTTTGCACAA AACCTGTGTT
16301	GCACCACTGT AGTCTTTAGC CCGCTTAGG GCTCCACCG CACTACAG GGTGAGTTATG ATGAGGTGTTA GACTGCTTG GACTGCTTG AGCAGGTCAA GTTGTGCGCA TCAGAAATGC GTGCTACTTG GTAGGTGAG GTGAGTGTTC GTGAGTATG TACTGCAT TCGGCTGCTC CTGAGCAAC TGGTCCGTT
16401	CGAGGCGCTC GTTGAATTTG CTTACCGAAA TGGGATATAG GATATGTTG GTTGTGCTCT GTACAGATTC ACCCGACAC CTAGCTTAAA GCGCGTAAAC GCTTCGCGAG CCGCTCAAC GATGCGCTTT GCGGTATTC CTGTACGACC GCAATGCTA CCGTCTCCG TTGGGTTGTG GATCGGATTT CCGGCTTTGT
16501	TTGCTGCGCG GCTTGTACCG TCGTAGAATA AGGCGCTCT AAGATGAG AAGTGTGCTG TCTGTGCTACT TGGCACCCAC CCGTGTATCG ATGCTATCCA CTGACGCGCC AGACGCGGCT CGAAGGTGCG AGGCTCTTT TCGCGCGCTA TTTTGTGCTC ACACCACTGA ACCGTGGGTG GCACGTGAG TACCATATGT
16601	AGCCGACGG ACTGGAAGT GTCTTGAAA AATGACCGT GGAACCTTAG GTGAGACCCG GACTGCGGC TCCNAGCGCA CCGCGGTTAG TTGTTCCACC GCGGCCCTGA TCCGCGTCC TCACTTCTA CAGAACCTTT TTTACTGCA CTTTGACTC GTTGTGACT TTGCGACCG CACAGAGGCG ATGTGAGACAC AATCGTCCCG GTTTGCTCTA
16701	GGGCGTGCAG ACCGTGACG TTTAGATACC CACTACAGT AGCAACATTA TTGCGACCG GACTCGGCG GTGCTCTCGG ACCGTGCTG TTTGCGAGCG CCACGAGT CCTGCAGTTC TGGCACTGC AAGTCTATG GTGATGTCTA TCGTGTGCT ATCGGTGCG GTGCTCTCGG GTGCTCTCGG GTGCTCTCGG GTTGTGCTG TTTGCGAGCG CCACGAGT
16801	GGGCTGCGCG ATCGCGCGCT CCGAGCGGTC GCGAGCGGCG GCTTGTGCTG GATGTGCTG GTGCTGCTG GTGCTGCTG GTGCTGCTG GTGCTGCTG GTGCTGCTG CCTGCAGCGC TACGCGCGCA AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC
16901	GGGCTGCGCG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC CCTGCAGCGC CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC GATATGCTC
17001	CACCTACCG CCGAGAGAC GAGCACTAC CCGAGCGCG GCGCGTGTGCG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC GTGATGTGCG GGTCTCTCTG CTGCTGTGAG ACCGTGTGCG TCGCAACAG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC
17101	GTGCGCAGCG TGGCTGTGCA AGAGGCGAG ACCGTGTGCG TCGCAACAG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC CAGCGGTCCG ACCGAGCGCT TCGTCCGTC TCGCAACAG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC GATATGCTC
17201	ATATGCGCT CAGCTGCGCG CTGCGTTTC CCGTGTGCG AGGTACGCG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC GATATGCTC TATACCGCGA GTGACGCGCG GAGCGAAGG GCGAGCGCG TCGTGTGAG AGGTACGCG CCGTGTGAG AGGTACGCG CCGCGAGCG GCTACTGCG GATATGCTC
17301	GGTGTGCG CAGCAGCGCG GCGCGCGCG GTGCGCGCT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT CAGCGCGCG GTGCGCGCG CAGCGCGCG GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT GTTGTGAGT
17401	GTGCGCGCG TTTGATGCTT GCGCTGTGAG GCGAGAGAC ACTGATTTA ATCAAGTTTC ATGTTGAAAA ATCAAAATTA AAGTCTGGA CTTCTACCGT CAGCGCGCTT ACGTGTGCA CCGGAGCGTC CCGTGTGAGT TCGTGTGAGT TCGTGTGAGT TCGTGTGAGT TCGTGTGAGT TCGTGTGAGT TCGTGTGAGT
17501	CGTGTGCG TGTGAGATTT TTGTAGATG GAGCATCA ACTTTGCTC TCTGCGCGG CGACAGCGCT CCGCGCGGTT CATGTGAGT TCGTGTGAGT CGGACCGAG ACATTGATA ACATGCTTAC CTTGTGTATG TGTGAGCGAG AGACCTGTG GCTGTGCGA GCGCGCGAA GTACCGCTTTG ACCGTCTAT

Figure 15K

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19301	AGACCTAATG	GGCCACAAAT	CTATCTGAA	CAGGCTAAT	TACATCTCT	TTATGATAT	CTATGATAT	ACACAGCAC	GGTAATATG
19401	TCTTGATAC	CCGTTGTTA	GATACGGTT	GTCTGATTA	ATCTGCTTT	AAATATCTA	GATATACAT	TGTTGCTGT	CCCATTTAT
19501	GGTGTCTGG	GGCCGCTGG	ATCTGCTTT	TAGCTGCTT	ATCTGCTTT	TGTTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT
19601	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT	ATCTGCTTT
19701	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
19801	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
19901	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20001	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20101	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20201	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20301	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20401	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20501	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20601	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20701	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20801	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT
20901	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT	TTCTGCTTT

Figure 1SM

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21001	Band II									
	Band II									
21001	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA
21101	AAATACAGTA	CCCGGCTGAG	TGCTGTGACC	CGATTTTGA	ACAGATATG	TTAGCTGCTG	TGCGATATC	GTACCTGATC	GTACCTGATC	GTACCTGATC
21201	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA	CGATGACAGA
21301	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA
21401	AAATACAGTA	CCCGGCTGAG	TGCTGTGACC	CGATTTTGA	ACAGATATG	TTAGCTGCTG	TGCGATATC	GTACCTGATC	GTACCTGATC	GTACCTGATC
21501	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA	CGATGACAGA
21601	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA
21701	AAATACAGTA	CCCGGCTGAG	TGCTGTGACC	CGATTTTGA	ACAGATATG	TTAGCTGCTG	TGCGATATC	GTACCTGATC	GTACCTGATC	GTACCTGATC
21801	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA	CGATGACAGA
21901	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA
22001	AAATACAGTA	CCCGGCTGAG	TGCTGTGACC	CGATTTTGA	ACAGATATG	TTAGCTGCTG	TGCGATATC	GTACCTGATC	GTACCTGATC	GTACCTGATC
22101	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA	CGATGACAGA
22201	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA
22301	AAATACAGTA	CCCGGCTGAG	TGCTGTGACC	CGATTTTGA	ACAGATATG	TTAGCTGCTG	TGCGATATC	GTACCTGATC	GTACCTGATC	GTACCTGATC
22401	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA	CGATGACAGA
22501	TTATGTCCTAT	GGGCGACATC	ACAGACCTTG	GCANAACT	TCTCT	ACTTCTGCTC	ACGGCTTACA	CATGACTTTT	GAAGTGTATC	CGATGACAGA

Figure 15N

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22601 ATCTTGCCCT TCGTAGACTG CTCTTTTACG GCGCTTTACG CTTCTTTGCT CTCTACATCC ATTCAATCA CGTCTCTCTT ATTATCATTA ATCTTTCCCT
 TAGAACCGGA ACCATCTGAC GAGTAAATCG CCGCGAGTGG TCAANAAGTGA GCGCTTTAGG TAAGTTAGT GCACGAGGA TAAATAGTAT TACGAGGCA
 22701 GTAGACACTT AAGCTGCCCT TCGATCTAG AGCTAGATC GCTCTTCTAC TCGTATCTTC TCGTATCTTC TCGTATCTTC TCGTATCTTC TCGTATCTTC
 CATCTGTGAA TTGAGCGGA AGCTAGATC GCTCTTCTAC TCGTATCTTC TCGTATCTTC TCGTATCTTC TCGTATCTTC TCGTATCTTC TCGTATCTTC
 22801 CAGGTACGCC TCGAGGAAAT GCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG
 GTCCATCTGG ACCTCTCTAG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG
 22901 CATACGCCG CCGAGCTTC CACTTGTCTA CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG CCGCTTTACG
 GTATGCCGCG GGTCTCGAAG GTGACCAT GTGACCAT GTGACCAT GTGACCAT GTGACCAT GTGACCAT GTGACCAT GTGACCAT GTGACCAT
 23001 CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT
 GGTACCGGAA GAGGTGCGT CTGTCTTACG CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT CCGTCCCTTT
 23101 CCGCATACCA CCGCTTCTTC ATTCTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 GCGTATCTGT CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23201 ACCATTCTTA CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 TCGTAAATCAT CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23301 TCGTCCGCGC ATTCTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 AGAACCGCG TTACTCTTTT AGCGGCGCG TCGAGTACC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23401 CCGTATACCG CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 GAGCTATCTG CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23501 GCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 GCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23601 AGAGGAGACG CCGTACCGCG CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 TCTTCTCTTC CCGTATCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23701 GAGAGGAGGA GTGATTTATG AGCGAGCC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23801 GCGAGCGGAA AGTCTCTCTG GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA GCGAGCGGAA
 CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 23901 GCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 24001 ACCCTCCGAA CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 TCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 24101 TTTTTCGAAA ACTGCGAGAT ACCCTATCTC TCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC
 AAGAGGTTT TCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC CCGCTTCTTC

Figure 15D

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24201	CGTGGTCTCA CGAGGTGCA AAGATCTTTG AGATTTTCTG AGT GAGGAG AATGCTGCTG CAAAGCTGCT CAAAGCTGCTT TTTGTCCTTT TACTTTTCAGT GGAGCGAGTT GTTTCACGCT TTTTAGAAGC TCCAGAGCC TTGATCTCTC TTGATCTCTC GTTTCAGGAG GTTTCAGGAG GTTTCAGGAG GTTTCAGGAG
24301	CTCTGGATGG TTGGTGGAG TCGAGGTGA CAGCGCTGT CTAGCTCTAC TAAATCTAG CATGTAGTTC ACCACTTTTG CCTAGCCGCG ACTTAAACCTTA GAGACTCTAC AACCACCTTG AGCTCCCACT GTTTCAGGAG CATGTAGTTC AATTTTCTCT GTAGCTCTAG TGGTGGAGAG CAGTGGCGG TGAATTTGGAT
24401	CCCCCAAGG TCTATGAGCAG AGTATGATGT GATCTGATGT TTTGGTCTCT GTTTCAGGAG CATGTAGTTC GAGAGGATG CAAATTTGCA AGACAAAGCA GAGGAGGCT GGGGGCTTCC AGTACTCTGG TCACTACTCA CTGCACTAGC ACCTGTAGCG GTTTCAGGAG CATGTAGTTC GTTTCAGGAG GTTTCAGGAG GTTTCAGGAG
24501	TACCCGCAAT TGGCAGCAG CAGCTAGGCG CTTGCTCTCA AAGTATGATG CTTGCTCTCA TGGAGGAGCG AGCCAAACTA ATGATGCGCG CAGTCTCTCT ATGGCGCTCA ACCGCTCTC GTGATCTCG CGACCGAGT TTTGCTCTCT GAGCGCTGCA ACCTCTCTCG TGGTGGAGT TACTTACCGCG GTTACGAGCA
24601	TACCGTGGAG CTTGAGTGA TCGAGCGTT CTTTCTCTAC CCGGATGTC AGCGTAGCT AGAGTAAACA TTGCACTACA CTTTCTCTCA GGGTACCTTA ATGGCACCTC GAACTCAGCT AGTCTGCCAA GAAAGCAGCT GGGCTCTAG TCGCTCTCTG TCTCTCTCTG AAGCTGATGT GGAAGCTCTT CCGGATGCT
24701	CGCCAGGCTT CCAAGATCTC CAAGGTGGAG CTCTGCAACC TGGTCTCTCA CTTTGGATTT TTGCAAGAA ACCGCTCTG CCAAAAGCTG CTTCATTTCA GGGTCCCGA CTTTCTAGAG GTTTCACCTC GAGAGCTTG ACCATAGCAT GAACTCTTA TCGCTCTCTT TGGCGAACC CTTTCTCTG CAAATTAAGT
24801	CGCTCAAGGG CGAGCGCGCG CGCGACTAG TCGCGACTG CGGCTGATCG AGCGCTGAC GCMAATGAT AAGATAGCA TGTGTAGCGT CTGCGCTTAC CCGCAAGCG TGGTCAAGAA CGAGTTTCCC GTTTCGCGCG GCGCTGATCG AGCGCTGAC GCMAATGAT AAGATAGCA TGTGTAGCGT CTGCGCTTAC CCGCAAGCG TGGTCAAGAA
24901	CGAGGAGTGC AACTCTAAGG AGCTGCAAG AACTGTAAG ACTGTAAG ACTGTAAG ACTGTAAG ACTGTAAG ACTGTAAG ACTGTAAG ACTGTAAG CTCTCTCAG TTGGAGTTCC TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT TCGAGCTCTT
25001	GACATCAATT TCGCGGAGCG CTTGCTTAA ACCCTGCAAC AGCTCTGCTC TCTGAGTCTC TCTGAGTCTC TCTGAGTCTC TCTGAGTCTC TCTGAGTCTC CTGTAGTAA AGGGGCTTGC GAGCTGATTT TCGAGCTCTC TCGAGCTCTC TCGAGCTCTC TCGAGCTCTC TCGAGCTCTC TCGAGCTCTC TCGAGCTCTC
25101	AGCGCTCAGG AATCTTCCCG GCGCTCTCT GTGCACTTCC TACCGACTTT GTGCCAATTA AGTACCGCGA ATGCCCTTCC CCGCTTCTCG GCGCTTCTG CCGTACCGCT TGGCGAGTCC TTAGAAGCGG CCGTGGAGCA CAGGTGAAGG ATCGCTGAAA CAGGGTAAAT TCAATGCGCT TACGGAGCGC GCGCAAGCC CCGTACCGCT
25201	CTTCTCTGCG CTAGCGCACT ACCTTGCTTA CCACTCTGAC AATATGAG AGTATGAGG CTGAGTCTG TTAGCTCTTA CTGAGTCTG ACTGTGCTG CAACTCTG CGAGAGCTC GATCTCTTCA TCGAAGCGAT GTTGAGACTG TATTTACCTTC TGCACCTGCG ACTGCCAGAT GACTCTCAGG TGCACAGCGAC GTTGTATATC
25301	ACCGCGGACC GCTGCTCTGT TTGCAATTCG CAGCTGCTTA ACGAAGTCA AATTTAGCTT ACCTTTGAGC TGCAGGCTCC CTGCGCTGAC GAAATGCTCT TGGCGCTGCG CGAGGAGCCA AAGCTTAAGC GTTGAGCAAT TCGTTTTCAGT TTAATAGCCA TGGAAACTCG AGCTCTCAGG GAGCGGACTG CTTTCTAGG
25401	CGCTCTCGCG GTTGAAGTCT ACTTCCGAGG TGTGAGCTG GCTTTACTTT GCGAATTTTG TACTTAGCA TACCAAGCC CACGAGATTA GGTCTTACGA CGCGAGGCCC CAATTTTJAG TGGGCGCGG ACACCTGCG CCGAATGGA GGGTTTAAAC ATGCACTCTT GATGCTGCG GTGCTCTAAT CCAAGATCTT
25501	AGACCAATCC GCGCGGCTTA ATCGGAGCT TACCGCTGCG GTTATTAGCG AGGCTGACAT TCTTGGCCA TTGCAAGCCA TCAAGCAAGC CCGCGCAAG TCTGCTTJAG GCGGCGGAT TACGCTTCCA ATGCGGAGG CATTAATAGG TACCGCTTGA ACGAAGCTTT ACGTTTCTG AGTTGTTCTG GCGCTTCTT
25601	TTTCTCTTAC GAAAGGAGCG GGGGTTTAC TTAGACCTCC AGTCTGCTGA GAGCTCTGAG CCAATCTCCC GCGCGCGCG CCGCTTCTG CCGCTTCTG AAGACGATG CTTTCTCTG CCGCGCAATG AACCTGGGCG TACGCGCGCT CCGCTGCTG GCGCTTCTG CCGCTTCTG CCGCTTCTG CCGCTTCTG

Figure 15P

[illegible]

Figure 1502

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27301 CCTCCGCGCC ACTATCCGGA TCATTTTAT CTTACCTTTG ATG GTAAA GCACTGCGG GACGCTAGC ACTGAATGTT AAGTGGAGAG GCAGGCGAAC
 GAGGCGCGG TGATAGCCCT AGTTAATATA GGATTGAAAC TGG TATTTT CCAAGCTGTC CTATGATATG TTACTTACAA TTCACTCTCT GGTCTCTTT
 27401 TCCGCTGAA ACACCTGTC CACTGTGCG CCACAAATG CTATTCTCTT GACTTCGCTG TCTTGAATTA CCAAGGATTC ATATCGAGAG
 ACAGGACTT TGTGACCCAG GTGACACCG CGGTGTAC GAAATCTGAC CTGACGCGC ATTAAGCTA GAAACTTAAC GCGCTCTTAG TATAGCTCTT
 27501 CCGGCGCAC GCGTCCGCG TTACGCGCA GCGAGACTT GCGGTATGCT CCGCTATGCT GCAATCGGCA ACTAAAGCTT CAATGGGTC GCGGGGAGC ATCAACTGCG
 GCGCGCGTG CCGACGCGG AATGCGCGT CCGTCTCGAA CCGCATCGG
 27601 CCTGTGTGTC TCACCTGTAT TTGCACTGT TTGCACTGT CTTAACCTG GATTACATCA AGATCTTGT TCGCATCTCT GCGTGAAGTA TATTAATATC AGAATTA:A
 GGBACAGAG AGTCACACTA AACCTTACCA GATTGCGAC CTAAATGAGT TCTAGAAACA ACGGTATGAG CACGACTCAT TATATTATG TCTTTAAT
 27701 ATTAATCGG GCTCTATG GATCTCTGTA AACGCGACG TCTTCACCGG CCGTAAGCAA CCGTACCTG CTTTACCTG TACTTTTAA ACCTCTCG
 TATATGAGCC CAGCATAGC GGTAGGACAT TTGCGGTGCG AGATGCGG CCGTTCGCTT GGTTCGCTT GGAATGAGC ATGAABATG TAGAGAGGA
 27801 CTGTGATTTA CAACGTTTC AACCGAGCG GATGAGTCT ACAGAGAAC CTTCTCGCTG GAGAGGCTG AGCTGATG TCAAGTACTC CATACAGAAA AACCCAGC TCTTACC
 GACACTAAT GTTGTCAAG TTGCTCTGCG CTACTCAGA TCGTCTCTTG GAGAGGCTG AGCTGATG AGCTGATG TCAAGTACTC CATACAGAAA AACCCAGC TCTTACC
 27901 CCGGACCTT ACAGTGTGCT CACCGCGCG TTACACACAC CTACCGCTG CTACCGCTG AGCTGATG AGCTGATG TCAAGTACTC CATACAGAAA AACCCAGC TCTTACC
 GCGCTTCCA TCTTCACCGA AGCTGATG GTGCGCGCG AGCTGATG AGCTGATG TCAAGTACTC CATACAGAAA AACCCAGC TCTTACC
 28001 AACAGAGGT GAGCTTGA NAACCTTAGG GTATTAGCG AAAGGCGAG CTACTGTG GATGAGGCTG GATGAGGCTG TCAAGTACTC CATACAGAAA AACCCAGC TCTTACC
 TTGCTCTCCA CTGGAATCTT TTGGAAATCC CTTAAATCG TTTCGCGCTG
 28101 TCAGCTTCT CTAGAAAGG GGTGCGGTT ATTCTCTGCT TTGTGATCT CTTTATCTT ATACTAACG TTCTCTGCT AAGGCTGCG GCTCTCTT
 AGTCCAAAGA GATCTTAGC CCAACCGCA TAAGAGAGC AACACTAGA GAATATAGAA TATGATGCG AAGAGAGGA TTCCGAGCG CCGACGAC
 28201 TCCACATTT GATTTATGT CAGCTTTTAA AACGCTGCG TCGCCACCA AGATGATG GTACATAATC CTAGGTTTAC TCAGGCTTGC GTACGCTTGC
 ACCTGTAAAC GTAAATACA GTGAAATAT TTGCGACCG TTGCGACCG AGCGTGGT TCTACTAATC CATGTATTAG GATCCAAATG AGTGGAGCG CAGTGGGT
 28301 AAGAGTCCA AAGAGTCCA TTTAAGGAG CAGGCTGTA ATGTTACAT CCGAGCTGA GCTAATCAGT CCAAGCTCT TATTAATATC ACCACAGN
 CAGTGGTGG TTTTCACTT AATTTCTC GGTGAGCAT TACATGTA TACATGTA CCGTCTGCT GATTTACTCA CCGTGGAGGA ATATTTTACG TCGTCTCTT
 28401 ATGAAGCT GATTTATG CACAAACA AATTTGCAA GTATCTGTT TATGCTATTT TCAAGCAGG TCACACTACA GATATATG TTACAGTTT
 TACTTTTGA CCAATAAGCG GTGTTTTT TTTAACCTT CATACAGCA ATACGATAA CCGTGGTCC ACTGTGATGT CTCATATTTAC AATGTCAAA
 28501 CCAAGGTAAA AGTCATAAA CTTTATGTA TACTTTTCCA TTTATGAAA TGTGAGCAT TACCAGTAC ATAGGCAAC AGTATAGTT GTGCGCGCA
 GGTCCCATTT TCAGTATTTT GAAATATCAT ATGAAAGGT AATATCTTT ACAGCTGTA ATGCTATAG TACTGTTTG TCATATTTCA CACCGTGGT
 28601 CAATATTTG TGGAAACAC TGGACTTTC TGTGACTG CTATCTAAT TACAGTCTC CATTGCTCT GTAGCTTACT CTATATTTAA TACAAAGCA
 GTTTTAAAC ACCTTTTGT ACCGTGAAAG AGGAGTAC GATAGATTA ATGTCACGAG GAAACAGGA CATGGAATGA GATATATTT ATGTTTCT
 28701 GACGAGCTT TATTGAGGA AAGAAATGC CTTAATTTAC TATGTTACAA AGCTAATTC ACCACTAAT GCTTACTGCG CCGTCTGCAA AACAAATTA
 CCGGTGAAA ATAACTCTT TTCTTTTACG GAATTAATG ATTCATCTT TCGATTACG TCGTATGTA CCAATGAGC GAAATGAGC GAAATGAGC TCGTCTGCAA
 28801 AAGATTAGC ATTAATTTA GATAGGAT TAAACCGCG GTCATTTTC TCTCTAATAC CATTCGCTG AACATTTGAC AGATATGAG TCTATGCGG ATATCTCTA
 TTTTCAATCG TAATATTAAT CTTATCTTAA ATTTGCGCG CCAATAGG AGGATTTATG GTTAGGGAC GTTAGTACTG AGATACAGC TATPACGAGT

Figure 15R

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28901 GCGCTACAC CTTGAAGTCA GCTTCTCTAG AGTGTAGCAT CTGACTTTTC CCAAGACCTG TCCCTCCGAT TTCTTCCAGT CCACATACAG CCACCCACCTC
 29001 CCGATGTTTG GAACCTCAGT CCGAGAGATC TACAGTCTTA GACTGAMACC GGTGCTGTAC AGGCGCCTA AACAGGCTCA GGTTCATGTC GCTTGGTATG
 29101 TACACAGAT GACACACACA ACCACACGCG CCGCGCTAC CCGACTTACA CCGACTTACA TATACACCCA ATATACACCCA TATACACCCA TATACACCCA
 29201 ATGCTCTCA CTGCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 29301 GACCCCTAC ACCACACACA GGTATGCTCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA
 29401 TATAGTCCA TCAATGCTCT ACACCCACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA ATATACACCA
 29501 ATATACAGGT AGTACACCA TGTGCTCTT TACTACTCTT TACTACTCTT TACTACTCTT TACTACTCTT TACTACTCTT TACTACTCTT TACTACTCTT
 29601 CATGATTCCT CCGATTTTCA TATTACTGAC CTTCTCTCTT CTTCTCTCTT CTTCTCTCTT CTTCTCTCTT CTTCTCTCTT CTTCTCTCTT CTTCTCTCTT
 29701 GTACTAAGGA GCTCAAAAT ATATATGACT GCGACACAC GCGACACAC GCGACACAC GCGACACAC GCGACACAC GCGACACAC GCGACACAC
 29801 GCGCTACAG TCTATTTCTT TTACGATTTT GTACCCCTCA CCGCTATCTT CCGCTATCTT CCGCTATCTT CCGCTATCTT CCGCTATCTT CCGCTATCTT
 29901 CCGAAGTCTC AGATAACGA AATGCTTAA CAGTGGAT GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC
 30001 GTGTGCTCTT TGCATATCTC AGACACATC CCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC
 30101 CACAGCGAA ACCTATAGAG TCTGCTCTT GCGCTATCTT GCGCTATCTT GCGCTATCTT GCGCTATCTT GCGCTATCTT GCGCTATCTT GCGCTATCTT
 30201 CTGCTGATTA TTGCACTCTT ATCTGCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 30301 GACGACTAAT AAACCTGCGA TACACCGAA ACACCGCTT GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC GCGATGAC
 30401 GCTACATGA AAAAGCGAT CTTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 30501 CCAATGTTACT TTTTCTCTT GAAAGCTTC GCGACATAT ACCTTAGT AGCAATATC ACAAGCTCT ATGCTAGAT CCGATGAC ATATAGGAT
 30601 CCTGACATTT GCGCTGACG CATATATCT CAGTACCTC CCGATGAC CCGATGAC CCGATGAC CCGATGAC CCGATGAC CCGATGAC
 30701 GGAAGCTGAA CCGACCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT GTATCTCTT
 30801 CCAGCCCATC AGCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT ACCCTCTCTT
 30901 GGTCTCTCTT TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG TCGAGCGGG
 31001 GGAATTTTCA CAGAGCGCG CCGCTGAGAA AGCGCGCGG CAGCGCGCG CAGCGCGCG CAGCGCGCG CAGCGCGCG CAGCGCGCG CAGCGCGCG
 31101 CCTTAATAT GTCTCTCTT GCGAGCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 31201 GCAAAAGCG GTCTCTCTT CCGTAAAGC AGCTCTCTT CAGCTCTCTT CAGCTCTCTT CAGCTCTCTT CAGCTCTCTT CAGCTCTCTT CAGCTCTCTT
 31301 CGTTTCTCTT ATGAAACCA GAGCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT TGTCTCTCTT
 31401 GAAATGCTG GTCTCTCTT CAGAAAGCG CATTACTCTT ACTCTCTCTT ACTCTCTCTT ACTCTCTCTT ACTCTCTCTT ACTCTCTCTT ACTCTCTCTT ACTCTCTCTT
 31501 CTTTAACCA CAGTACCA CTTCTCTCTT GTATCTCTT TCACTCTCTT TCACTCTCTT TCACTCTCTT TCACTCTCTT TCACTCTCTT TCACTCTCTT TCACTCTCTT
 31601 CTTCTCTCTT TTTTCTCTT CTTACATCTT TTTTCTCTT TTTTCTCTT TTTTCTCTT TTTTCTCTT TTTTCTCTT TTTTCTCTT TTTTCTCTT
 31701 GAGAGCTGCG AATAATCTT GAGACCGCA GAGTCTCTT AATAAGGTA AATAAGGTA AATAAGGTA AATAAGGTA AATAAGGTA AATAAGGTA

Figure 155

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30401 AATTTCTGT CAGTTTATT CAGCAGACC TCTTTGCTT CCTC CAGCT CTGTATTGC AGTTCTCTC TGGTGCAMA CTTCCTCAC ATCTAATAG
 TTTAAGACA GGTCAATATA GTGGTGTGG AGTAAGGGA GAGAAATCCA GATCATACAG TTGACAGAG GAGACAGCTT GAGAGAGTG TTAGATTAC
 30501 GAATGTCACT TTCTCTCTGT TCTGTCTCAT CCGCAGCCAC TATCTTCTATG TGTATTGAGA CAGATACCTT AGATACCTCT TCAACCCCTT
 CTACAGTCA AGGAGGACA AGGACAGGTA GAGATGTGTT ATAGACTATC CACTCTCTCT TCTGCTGCG TTCTGCGAGA CTCTATATGA AGATTGGG A
 30601 GTATCCATAT GACACGAMA CCGGTCTCTC AGCTGTGCTT TTTCTTACTC CTCTCTTGT ATCTCCCAAT GGGTTTCAG AGATGCTCCC TGGGTACT :
 CATAGGTATA CTGTGCTTT GCCCAGGAG TTGACACGGA AAGCATGAG GAGGGAACA TGGGGTTTA CCCAAGATTG TCTGAGGGG ACCCCATGAG
 30701 TCTTTGCCC TATCCGACC TCTAGTTACC TCCATGACA TCTTTGCTT CAAATGCG CAACTCTCT CTCTGAGGA GGCCTGAC CTTACTCT:
 AGAAGGCG ATAGCTTGG AGATCAATGG AGTTACCTT AGTACCGA GTTTTACCG TTCTCCGAGA GAGACCTGCT CCGGCTGTG GANTGAG :
 30801 AATATGATC CACTGTGAC CCACCTCTCA AAAAAACCA GTTAAATATA AACTGTGAAA TGTCTTACCC CCTCACAGTT ACCTCAGAG CCTTACTCT
 TTTTACATT GTGACACTGG GTTGTGAGT TTTTGTGTT CAGTTGTAT TTGACCTTT ATAGACTGZ TGGAGTCTT TGGAGTCTT GGGATTGACA
 30901 GGTGCTGCC GCACCTTAA TGGTCCGCG CAACACACT ACCATGCAT CACAGCCCC CTAACCTGT CAGGACTCCA AACTTAGCAT TCCACCCCA
 CCGACGCGG CCGGAGATT ACCAGCCCC GTTGTGAG TGTAGCTTA GTGTCCGCG CGATTGCGAC GTCTGAGGT TTGAATGCTA AGGTGGGT P
 31001 GAGCCCTCA CAGTGTGAGA AGGAAGCTA GCTCTGCAA CACAGCCCC CTAACCTGT ACCATGCAT TGTACTGCTT TATCTGCTT TACCCCC
 CTTGGGAGT GTACAGTCT TCTTTGAT CCGGAGCTT GTATGCTGG GGAGTGGTG TGGTATCTT CATGGMATG ATAGTGACGG AGTGGGGA
 31101 TAACTACTG CACTGTAG TTGGCATG ACCTGATG TGAACCTT CCGTAAATA TGTATTACT CTCTGATCT TGTATGATG CCCCAGGAA ACTTACTT
 31201 AGGCACTCA ACATTTGA CCGTAGCAC TGGTCCAGT TGTGCTGCT GGTAACTA ATATACTTCA ATATGACTG GAGCTTGGG TTTTGTCTA
 TCTGTGAT TTGTGAACT GGCATGCTG ACCAGTCTA CACTGATAT TATTATGAG GAACTGTG TTTCTATGAC CTGGAGACC AATCTAAT
 31301 CAGGCAATA TGCATTTAA TGTAGCAGGA GACCTNAGG TGTATCTCA AAGAGGCG CTATACTTG ATGTTAGTTA TCGTTTGTAT GCTCAAAAC
 GTTCCCTTAT AGTTGATTT ACATGCTCT CCTGATCTT ACTAAGAT TTTGTCTGG GAATATGAC TACATCAAT AGGCAACTA CGATTTT
 31401 AACTAATCT AAGACTAGGA CAGGCCCC TTTTATATA CTCAGCCAC AACTTGGATA TTAAGTACA CAAGGCTT TACTTGTTA GAGTTCAA
 TTGATTTAGA TTTGATCT GTCCGGGAG AAAAAATTT GAGTGGGT TTGATGTT ATTTGCTT ATGACCAAT GTGAAATTT
 31501 CAATTCAMA AGCTTGAG TTAACCTAG CACTGCCAG GGTGTGAT TGTGCTAC AGCATAGCC AGTATGCGT TGAATTTCTT
 GTTAAGGTT TTGAACTCC AATGGAATC CCAACTACA AACTGGATG TCGTATGCG TAATTAGCTC CTCTACCGA ACTTAAACCA
 31601 TCACTTAATG CACCAACAC AATCCCTC AAAAAAAA TTTGCAAGG CCTAGATTT GATCAACAA AGCTATGAT TCTTAACTA GGAATCTGT
 AGTGAATTAC GTGTTTGT TTTAGGCG TTTGTGTTT AACCTTACC GGATCTTAA CTATGTTGT TCGATACCA AGATTTGAT CTTGACCTG
 31701 TTAGTTTGA CAGCAGCT GCAATACG CCGTAATGTC AATATGAT TTTATTTA TTTGATGAA ACACCTGCG TGGTGGGT AGGATTTGA CATCTGATT
 AATCAAACT GTGTGTCA CCGTAATGTC AATATGAT TTTATTTA TTTGATGAA ACACCTGCG TGGTGGGT AGGATTTGA CATCTGATT
 31801 TCCAGAGAA GATCTAAG TCACTTGT TTTAACAAA TGTGCTGTC AATACTTGC AATCTGCTG TTAAGGCG TTAAGGCG TTTGCTGCA
 AGCTCTCTT CTACGATTG AGTGAACA GATTTGTTT ACACCTGTC TTTATGACG TTTATGACG ATGTCACAA CAAACCGG AATTTCCGTC AAACGAGG
 31901 ATATCTGAA CAGTTCAAG TGTCTATCTT ATTAATAGAT TTGAGGAA TGTAGTCTA CTAAACAT TCTTCTGGA CCCAGATAT TAACTTTA
 TATAGNCTT GTCAAGTTT ACAGTAGA TATATTTCA ACTGCTTT ACTCAGAT GATTTGTTA GGAAGNCT GGTCTTATA ACCTTGAAT
 32001 GAAATGAGA TCTTACTGA GGCACACT ATACAAAGC TGTGTGATTT ATCTTACG TATCACTT TCCAAATCT CAGGTAAAA CTGCAAAAG
 CTTTACTCT AGATGACTT CCGTCTGGA TATGTTTGG ACACCTAA TACGATGG ATGTTTGA AGTTTGA GTGCCATTT GACGTTTTT

Figure 15T

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32101 TAACATGTC AGTCAGTTT ACTTAACCG AGCAAAACT AAACCTTATA CACTAACAT TACACTAAAC GGTACACAGG AACAGBAGA CACAACCA
ATTGTAACAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG
32201 AGTCAGTACT CTATGTCAT TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG
TCACTATGTA GATACAGTAA AGTACCTCG AGCAACCGG TGTATATATA ATTACTTAT AAACCTTATA CACTAACAT TACACTAAAC
32301 AATAAGAAAT GGTTCGTTT TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG
TTATTTCTTA GCAACACAA TCAACAGTTG CACAAATAT TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG TCAATTCAG
32401 GCTTATACAG ATCAGCTAC CTTAATCANA CTCACAGAC CTTAGTATTC AAACCTTATA CACTAACAT TACACTAAAC GGTACACAGG
CGATATATG TATGTCGAT GATTCGATG GATTCGATG GATTCGATG GATTCGATG GATTCGATG GATTCGATG GATTCGATG GATTCGATG
32501 GCTGCTCTTA AAACCTTATA TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT
CGACCGAAT TTTTGTAGT ATAGTACCA TTTTGTAGT TTTTGTAGT TTTTGTAGT TTTTGTAGT TTTTGTAGT TTTTGTAGT TTTTGTAGT
32601 ATAACTTCC CGGCGAGTC ACTTAAGTC ATGTCGCTG TAAATTCAG TACAGCACA GTTCAGCAG TCGTGTCTG ACACAGGTT GAACTTCCCG
TATTTGAGG GCGCTGCG TAAATTCAG TACAGCACA GTTCAGCAG TCGTGTCTG ACACAGGTT GAACTTCCCG GAACTTCCCG
32701 AAGTCCAGC CTACATGCG GTAGATCAT ATGTCGCTG CAGGATAGG GCTTCGCTG CAGGATAGG GCTTCGCTG CAGGATAGG GCTTCGCTG
TTCAGCTCG GATTCAGCT CATCTAGTA TTACAGCTA TTACAGCTA TTACAGCTA TTACAGCTA TTACAGCTA TTACAGCTA TTACAGCTA TTACAGCTA
32801 CCTGCAAGAA TACACATGCG CAGTGTCTG CTACAGCTG ATTCAGCTG CCGTACCAT AAGCTTCTT GTCCTCGCG CACAGCAGG CACCTGAT
GAGCTGCTT ATGTCGCTG GTACAGCAG GATTCGCTG TAACTGCTG TAACTGCTG TAACTGCTG TAACTGCTG TAACTGCTG TAACTGCTG TAACTGCTG
32901 TCACTTAAT TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA
AGTGAATTTA GTGTCGCTG TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA
33001 AAGCTGCTG GCTATCATAC CACAGCACA GGTAGATTA GTTCGCTG TACAGCACA TACAGCACA TACAGCACA TACAGCACA TACAGCACA
TTGCTGCTG CCGTATGAT GTGTCGCTG CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT
33101 CAGCAGCTC GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
GTGTCGCTG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
33201 AAGCTGCTG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
TCCCTGCTG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
33301 GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG CCGCTATG
GAGCTATG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
33401 AAGCTGCTG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
TTGCTGCTG CCGTATGAT GTGTCGCTG CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT CCGTATGAT
33501 GAGCTATG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG
CCTGCTATG GGTATCATAC TAACTGCTG ATTAACATG GCGCTATG CCGCTATG TAACTGCTG ATTAACATG GCGCTATG CCGCTATG

Figure 15U

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33601	CTGTAAACAA ACCAGTGG GGGGTGACAA ACAATCTCC GTCTCCGTC TTCCGCTTA GATCCCTG TGTAGTAGT GTATTATATC CACTCTCTA
33701	GACTTCGTT TGTCCACGC CCGCAATCT TGTCTACAGC CAGATCTCC AGTGGGCTT CATACAGAC ACATATCAA GATCATATG GTGAGAGAT
33801	AAGCATCCAG GGGGGGGG GCTTCGCTT CTATCTAAC TCTCTACG CAGATCTCC TATACATATC CACCACGCA GATTAAGCCA CACCCAGCC
33901	TTCTGATGAT CCGGGGGG CCAAGCCCA GATAGATTC AGCAAGTAC GGGGACGG ACTATTATG GTGGTGGCG CTATTATCCG GTGGTGGG
34001	ACCTACCAT TGTCTCTGG AGTCACAC GGTAGATTC GGAAGACCT GATAGACAT GTTTTTTT ATATTATCCA AGATTATCCA AATCCCTA
34101	TGGATCTGTA AGCAAGAC TCAATCTG GCTCTCTCC CTTCTCTG CTTCTCTG CTAATATGTA CAAATATGTA AATAAGTTT TCTAATAGCT TTTGGAGTT
34201	ATGAGATCT ATTAACTGAA CCGCTCTCC TCCGTGGG TGTCTMACT CTACAGCAA AGAACAGTA ATGCCATTT ATGCCATTT TAAATATGTT CACATATGCT CACATATGCT
34301	TACTTCTAGA TAAATCACTT GGGGAGGCG AGGCCAGCC ACCGATTTT GATGTCGTT TCTGTCTAT TACGGTAAAC ATCTATAC ATCTATAC
34401	TCCAAAGGC AAGGGGCT CAGGTCGAG TGGAGTAA GGTAAATCC TTCAAGTGA ATCTCTCTA TAAACATTC TAAACATTC AGCACCTTCA ACCATGCCA
34501	AGGTTTTCCG TTGCGCGGA GTGAGGTTT ACTGCAAT CCGATTTCC ATATTCTG ATATTCTG CCGCATTTG TATATTCTG CCGCATTTG TATATTCTG
34601	AATAATCTC ATCTGCCAC CTCTCTATA TATCTTAAG TATCTTAAG ATATTCTG CCGCATTTG TATATTCTG TATATTCTG TATATTCTG TATATTCTG
34701	CAOCTCAG CAGGAACTA TGAATGCAA AATTCAGTT CCTCAGAC CTGTATAA TTCAAAAGCG TTAACATTAAC GAAATATTAAC CAAATATTAAC CAAATATTAAC
34801	GTGGAATTC GTGCTTAGT ACTAAGCTT TTAAGTCCA GAGATGCTG GAGATGCTG GAGATGCTG GAGATGCTG GAGATGCTG GAGATGCTG GAGATGCTG
34901	GGTCCCTTC CAGGCGAG TGAACATA CTCTCAGCT TGCAGGCT GCGGCGCT TGCAGGCT TGCAGGCT TGCAGGCT TGCAGGCT TGCAGGCT TGCAGGCT
35001	CCAGGAGGC GTCCCGTCC ACTTCTATA GCACTCCAG ACCTCCCTG TCCGCGCT TCCGCGCT TCCGCGCT TCCGCGCT TCCGCGCT TCCGCGCT TCCGCGCT
35101	TATGACACG ATACTCGAG CTATCTAAC CAGGCTAGC GGTATGAG CCGATGAG CTGTGCTG GGTATGAG CCGATGAG CCGATGAG CCGATGAG CCGATGAG
35201	ATACTGTCC TATGAGCTC GATACGATT GTGCTATCC GGTATGAG CCGATGAG CCGATGAG CCGATGAG CCGATGAG CCGATGAG CCGATGAG CCGATGAG
35301	GGCAAGCCT CCGGCAAAA AGAAGCACA TGTATGCT TGTATGCT TGTATGCT TGTATGCT TGTATGCT TGTATGCT TGTATGCT TGTATGCT TGTATGCT
35401	CCGTTTCCGA GCGGTTTT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT
35501	TCTCMAACAT GTCTGCGGT TCTGCTATA ACACAAATA AATTAACAA AATTAACAA AATTAACAA AATTAACAA AATTAACAA AATTAACAA AATTAACAA
35601	AGATTTGTA CAGACGCTA AGACGCTAT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT TGTGTTTT
35701	ATAGCATAA GACGACTAC GCGATGCG CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT
35801	CTGCTGATG CTGCTGATG CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT CCGTACGCT
35901	TCATATATTA AGACTGCTA MACATATC GTTGCTAGT CAACTAATG TAACTAATG TAACTAATG TAACTAATG TAACTAATG TAACTAATG TAACTAATG
36001	AGATATACAT TCTGAGCCAT ACAGCCGCTA TACCTGCTA TACCTGCTA TACCTGCTA TACCTGCTA TACCTGCTA TACCTGCTA TACCTGCTA TACCTGCTA
36101	TCTGTTGTA TGTGCGGCT ATCTGCTA TGTGTTGTA TGTGTTGTA TGTGTTGTA TGTGTTGTA TGTGTTGTA TGTGTTGTA TGTGTTGTA TGTGTTGTA
36201	TCCGCTTCA GATCACTA CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC CAGGCTCC
36301	AGGCGAGCT CTGTTGCTAT GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG GTGCGGAG
36401	GGCACAGCT CAATCAGTCA CAGTGTAA AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG AAGGCGAG
36501	CCGTGGTGA GTTAGTCA GTACATTT TTCCCGTTC ACCTCTGCT CCAATATG CCAATATG CCAATATG CCAATATG CCAATATG CCAATATG CCAATATG
36601	CCAGAAAC CCGACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA CTTACGCTA
36701	GGGTCTTTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT GGTGCGCTT

Figure 15V

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35301 CATTTTTARGA AACTACAAAT TCCACACACA TACAGTTTAC TCCGCTCTTA AACTAGCTC ACCGCGCCCG TTCCACAGCC CCGCGCCACG TCACAAACTC
GTAAATTTCT TTGATGTGTA AGGGTTGTCT ATGTTCATATG AAGCGTGAAT TTGATGACAG TTGAGCGCGC AGGGTTCGCG AGGGTTCGCG AGTGTTCGAG

35401 CACCCCTCTA TTATCATATT GCTTCAATC CAAATNAGG TATATTATAG ATCATGTAA TTANGATTC GGATCTGCGA GGCATGCTG GATGCTCTT
GTGCGGAGT AATGATATTA CCGAATTTAG GTTTATTTCT TACTACAAAT TACTTTTATG CTTATACGCT GGCATCTGAC CTACCGGAGG

35501 CCGATTATGA TTCTTTCTCG TTCCGCGCGC ATCGGATTC CCGGTTTCCA CCGCTATGGA TAGATATGGA CAGCTTCAGG CAGCTTCAGG
CGGTAAATCT AAGAAGAGCG AAGCGCGCG TAGCCCTAGG GCGGTAAGCT CCGCTACGAC ATCTACTGCT GGTAGTCCCT GTGGAAGTTC

35601 GGCAGCAAA GGCAGGAA GCTTAAAGG CCGCTTCTT GCGCTTCTT CATATGCTTC GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT
CGGTCTTTT CCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT

35701 GAGTTCGCGA AACTGACAG GACTATAAG ATACAGCGG TTTCGCGCG TTAGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT CCGCTTCTT
CTCACCGCT TTGCGCTGTC CTGATATTTT TATGCTTCTT TATGCTTCTT TATGCTTCTT TATGCTTCTT TATGCTTCTT TATGCTTCTT TATGCTTCTT

35801 TTCTCCCTTC GCGAAGCGTG GCGCTTCTT ATATCTAGG CTGTAGCTAT CTGATTTTGG TGTAGGTGCT ACATCCAGCA TCCGCTCTT
GACAGCGGA AAGAGCGAG CCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT

35901 TGCACCAACC CCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT
ACGTGCTTGG GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT

36001 CACTGCTTAC AGATTATGA GAGCGAGTA TGTAGCGCTT GCTAGAGCTT TCTTGAAGTG GTGCTTCTT TCTGCTTCTT TCTGCTTCTT TCTGCTTCTT
GTGACCAATG TCTTAATCT CTGCTTCTT ACATCGCGA GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT

36101 ATCTGCGCTC TGTGAGCGC AGTTACTTTC GCGAAGAGAG TTGCTTCTT TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC
TAGACCGGAG AGCACTTCTT TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC

36201 TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT TAGCGCGGAT
TGTGCTGCTA ATGCGCGCTT TTTTCTGCTA GAGTCTTCTT AGTAAGCTAG TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC

36301 TTGCTGCTAG AGATTATCA AAGGATCTT CACTAGATC GTTGATCTAG GAAATTTAG TTAGATTTCA TATATCTCA TTTGAACGAG ACTGTCAATG GTTACGATTT
AAACCACTAC TCTTAATGTT TTCTCTAGAA GTTGATCTAG GTTGATCTAG GTTGATCTAG GTTGATCTAG GTTGATCTAG GTTGATCTAG GTTGATCTAG

36401 TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTGCTTTC ATCCATAGTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT
AGTCACTCCG TGTATAGAT CCGTAGACAG ATTAAGCAAG TAGGTATCTAA CCGACTAGG GCGACTAGG GCGACTAGG GCGACTAGG GCGACTAGG

36501 TGGCGCCAGT GCTGCAATGA TACCGCGAGA CCGACCTCA CCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT GCGCTTCTT
ACCGCGTCA CCGCTTCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT ATGCGCTT

36601 CCGTCAACTT TATCCGCTC CATCCAGTCT ATTAATGTTT CCGCGGAGG TTAGTATGTT AGTTGCTTCTT TTAGTATGTT TTAGTATGTT TTAGTATGTT
GAGCGTTTAA ATAGCGGAG GTAGCTCAGA TAATTAACNA CCGCTTCTT ATCTATCTA TCAAGCTTCT TCAAGCTTCT TCAAGCTTCT TCAAGCTTCT

36701 CTACAGCAT CCGTGTGTA CCGTGTGTA TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC TGTATCTGTC
GATGTCTGTA GACCAACAT CCGAGCAATG AACCATCTG AAGTATGTC AGCGCAAGG TTGCTTCTT TTGCTTCTT TTGCTTCTT TTGCTTCTT

36801 AAGAGCGTT AGCTCTTCT GTCCTGCTT CCGTCTCAGA AGTATGTTT CCGGATGTTT ATCACTCATG GTTATGCGAG CACTGCTTAA TTCTTCTTCT
TTTCTGCGAA TCGAGGAGC CAGGAGCTT CCACTATCTT TCACTTCAAC TGTATGCTTAC TAATACCTT CCACTATCTT TAATACCTT TAATACCTT

36901 GTCATGCCAT CCGTAGATG CTTTCTGTC ACTGCTGAT ACTTACCA GCTATCTGTA TAATGCTTCA TGTGCGGAC GATTTCTCTT TGTGCGGCT
CAGTACGCTA GCACTTCTTAC GAAAGAGAC TGAACCTTCA TGAATGCTT CAGTATGACT CTATATCAT ATGCGCTTCTT CAGTATGACT CAGTATGACT

Figure 15W

pMRAd5qag MERGB2

37001 CACACGGGA TAATACCGG CCACATACCA GAACCTTAA AGT ATCATE ATTGAAAC GTCTTCGG GCGAAACTC TCACGACTC TACTCTCTTT
 GTTGTCCCT ATTATGGGG GGTGTATCTT CTTCGAATTT TAA GACTAG TAACCTTTG CAAGAGGCC CCTTTTGAG AGTTCCTAGA ATGCGACAA
 GAGATCCAGT TCGATTGAC CCACTCTGGC ACTCAATCA TCTTAATAT TTTTACTTT CACCTGCTT TCTGGGTGAG GAAACACAGC AGCGCAANT
 CTCTAGGTCA AGCTACATTG GGTGAGCAGG TGGGTGACT AGAATCTATA GAAATGAAA GTCTGTGCA AGACCCACTC GTTTTGTCC TTCCCTTTTA
 GCGCGAAAA AGGGAATAAG GCGGACACCG AATGCTTGA TACTATAGT CTTCCTTTT CAATATATT GAAGCATTTA TCAGGCTTAT TCTCTCATC
 CCGCGTTTTT TCCCTTATTC CCGCTGTGC TTACAACTT ATCAATATCA GAAGAAAA GTTATATAA CTTCGTAAAT AGTCCCAATA ACAGAGTAC
 GCGGATACAT ATTGATGCT ATTTAGAAA ATAAACAAAT AGGCTTTCG CCAACATTC CCGCAAGT GCCACCTGAG GTCTAAGAAA CCATTATTA
 CCGCTATGTA TAACTTACA TAAATCTTTT TATTGTTA TCCCANAGC GCGGTAAAG GGGCTTTCA CCGTGGACTG CAGNTCTTT GGTAAATAA

37101

37201

37301

37401 CATGACATTA ACCTATAAA ATAGCGTAT CACGAGGCC TTTCGTCTTC AGCAATGGA TCGCATTTCT TAAT (SEQ ID NO: 27)
 GTACTGTAAAT TGGATATTTT TATCCGATA GTGCTCGGG AAGCAGAG AGTCTTAAGCT AGCCTTAAGA ATTA (SEQ ID NO: 28)

Figure 15X

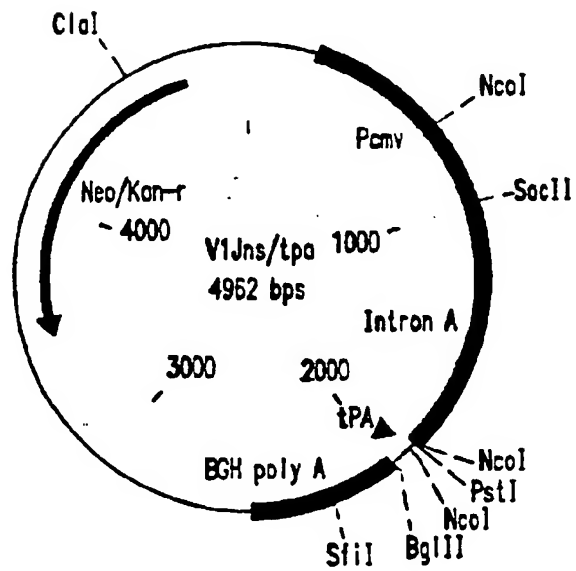
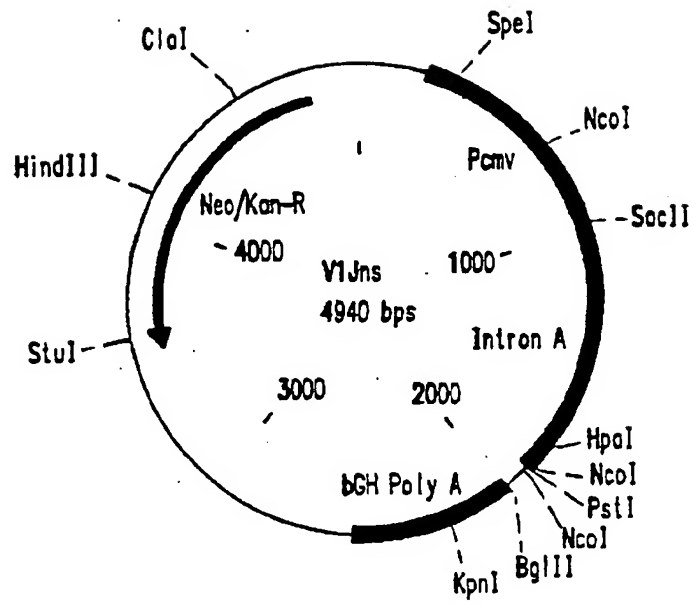


FIGURE 16

AGATCTACCATGGCCCCATCTCCCCATTGAGACTGTCCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA
 Bg/II MetAlaProIleSerProIleGluThrVolProVolLysLeuLysProGlyMetAspGlyProLysVolLy
 1 10 20
 GCAGTGGCCCCGTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuVolGluIleCysThrGluMetGluLysGluGlyLysIleSerL
 30 40 50
 AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCATCAAGAAGAAGGACTCCACCAAGTGGAGGAACCTGGTG
 ysIleGlyProGluAsnProTyrAsnThrProVolPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVol
 60 70
 GACTTCAGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluVolGlnLeuGlyIleProHisProAlaGlyLeuLysLy
 80 90 100
 GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG
 sLysLysSerVolThrVolLeuAlaVolGlyAspAlaTyrPheSerVolProLeuAspGluAspPheArgLysTyrThrA
 110 120 130
 CCTTCACCATCCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC
 loPheTnrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGly
 140 150
 TCCCCTGCCATCTTCCAGTCCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleVolIleTyrG
 160 170 180
 GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
 nTyrMetAlaAlaLeuTyrVolGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL
 190 200 210
 TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCCCTGTGGATGGGCTATGAGCTGCAC
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis
 220 230
 CCGACAACTGGACTGTGCAGCCCATTTGTGCTGCCTGACAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
 ProAspLysTrpThrVolGlnProIleVolLeuProGluLysAspSerTrpThrVolAsnAspIleGlnLysLeuVolG
 240 250 260
 CAAGCTGAAGTGGGCCTCCCAAATCTACCCCTGGCATCAAGGTGAGGCAGCTGTGCAACCTGCTGAGGGGACCAAGGCCC
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysVolArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL
 270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGAGATCCTGAAGGAGCCTGTGCAT
 EuThrGluVolIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis
 300 310

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA
 GlyVolTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGGCCACACCAATGATGTGAAGCAGCTGA
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT
 350 360 370

CTCAGGCTGTGCAGAAGATCACCAGTGAAGTCCATTGTGATCTGGGCAACACCCCAAGTTCAAGCTGCCCATCCAGAAG
 hrGluAlaVolGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe
 400 410 420

GGTCAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTTGTTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
 uVolLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGCAGGCAGAAGGTGGTGACCTGACTGACACCACCAACCAG
 luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC
 LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG
 aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG
 510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCACAAGGCCATTGGGGCAATGAGCAGGTGACAAGCTGGTGTCTGCTGGC
 luLysValTyrLeuAlaTrpVolProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly
 540 550

ATCAGGAAGGTGCTGTTCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAAGTGGAGGGCTAT
 lleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCTCCTGTGACAAGTCCCAGCTGAAGGGGAGG
 tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA
 590 600 610

CCATGCATGGGAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG
 lAlaMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal
 620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT
 AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
 640 650 660

GAAGCTGGCTGGCAGGTGGCTGTGAAGACCATCCACACTGCCAATGGCTCCAATTCACTGGGGCCACAGTGAGGGCTG
 uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA
 670 680 690

CCTGTGGTGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCACTCCAGGGGGTGGTGGCTCCATGAAC
 lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn
 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTTCT
 LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle
 720 730 740

CCACAACCTCAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATGCCACAGACATCC
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGCTGTACTACAGGACTCCAGGAADCCCTGTGG
 lThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
 780 790

AAGGGCCCTGCCAAGCTGCTGTGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGGTGGCCAG
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr
 800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGCCAAGCAGATGGCTGGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx
 830 840 850

AAAGCCCCGGCCAGATC (SEQ ID NO: 3)
 Xx Bg11 (SEQ ID NO: 4)

FIGURE 17C

FIGURE 18

WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT	-42
OPT	- ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC	
	M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT	-84
OPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC	
	T V R E R M R R A E P A A D	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC	
	R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC	
	V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC	
	N T A A T N A D C A W L E A	-70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG	
	Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC	
	P L R P M T Y K G A V D L S	-98
WT	- CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC	
	H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC	
	S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC	
	T Q G Y F P D W D N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG	-462
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG	
	P G I R F P L T F G W C F K	-154
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA	-504
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG	
	L V P V E P E K V E E A N E	-168
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG	-546
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC	
	G E N N C L L H P M S Q H G	-182
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC	-588
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC	
	I E D P E K E V L E W R F D	-196
WT	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG	-630
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC	
	S K L A F H H V A R E L H P	-210
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)	-651
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9)	
	E Y Y K D C (SEQ ID NO:10)	-216

FIGURE 19B

V1Jns/nef *PstI* *BglII*
 CATGGGTCCTTTTCGAGTCACCGTCCTTGAATCTGCCACC ATG GGC GGC AAG TGG TCC MAG AGG TCC GTG CCC
 M G G K W S K R S V P

. CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 38)
 H P E Y Y K D C * (contained within SEQ ID NO: 10)

V1Jns/nef(G2A.LLAA)

PstI *BglII*
 CATGGGTCCTTTTCGAGTCACCGTCCTTGAATCTGCCACC ATG GGC GGC AAG TGG TCC MAG AGG TCC GTG CCC
 M A G K W S K R S V P

. CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 39)
 H P E Y Y K D C * (contained within SEQ ID NO: 14)

V1Jns/tpanef & V1Jns/tpanef(LLAA)

PstI *BglII*
 CATGGGTCCTTTTCGAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG
 M D A M K R G L C C V

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ALC ICC TCC MAG AGG TCC GTG CCC
 L L L C G A V F V S P S E I S S K R S V P

. CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 40)
 H P E Y Y K D C * (contained within SEQ ID NO: 16)

FIGURE 20

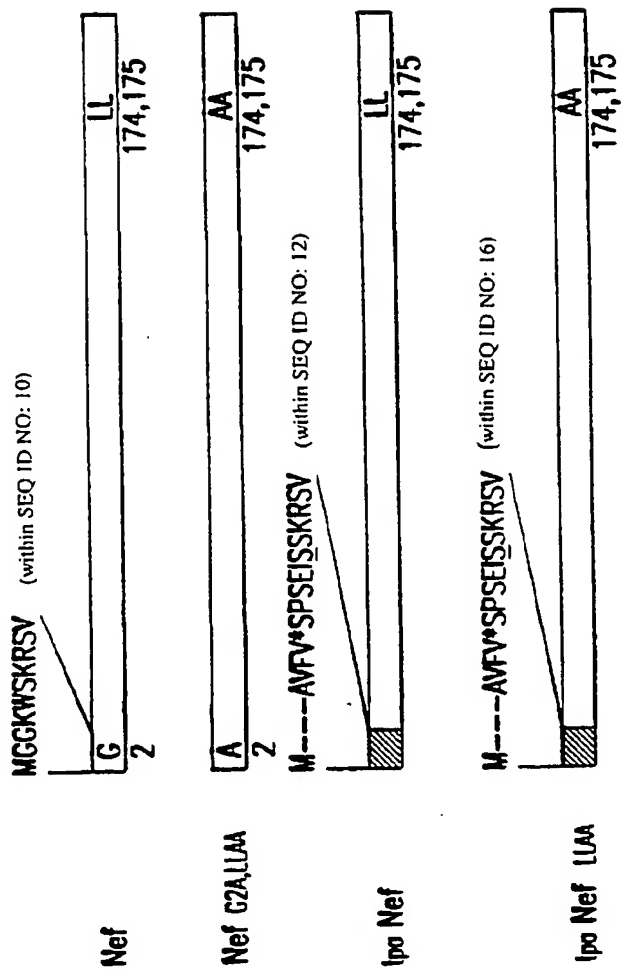


FIGURE 21

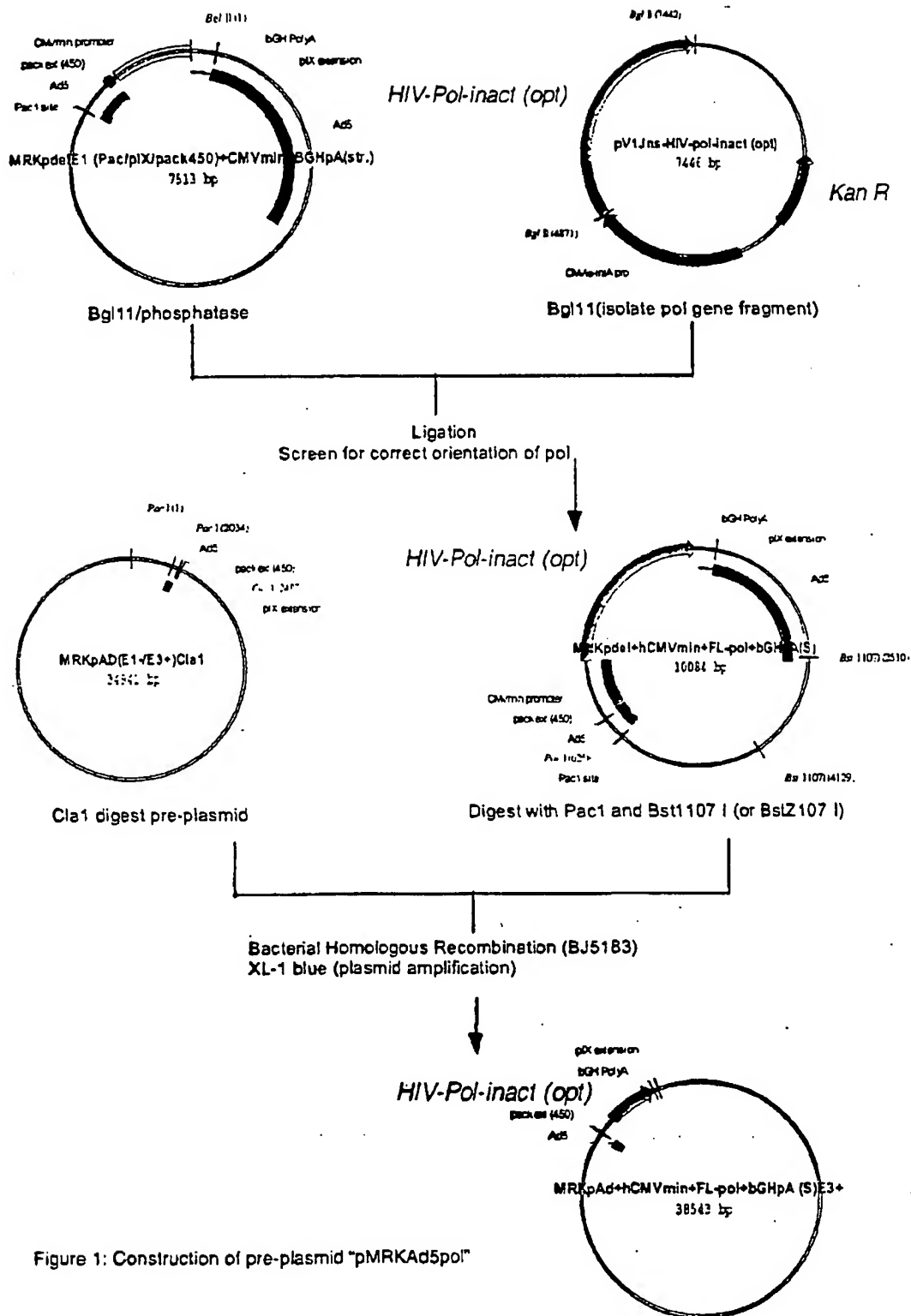


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

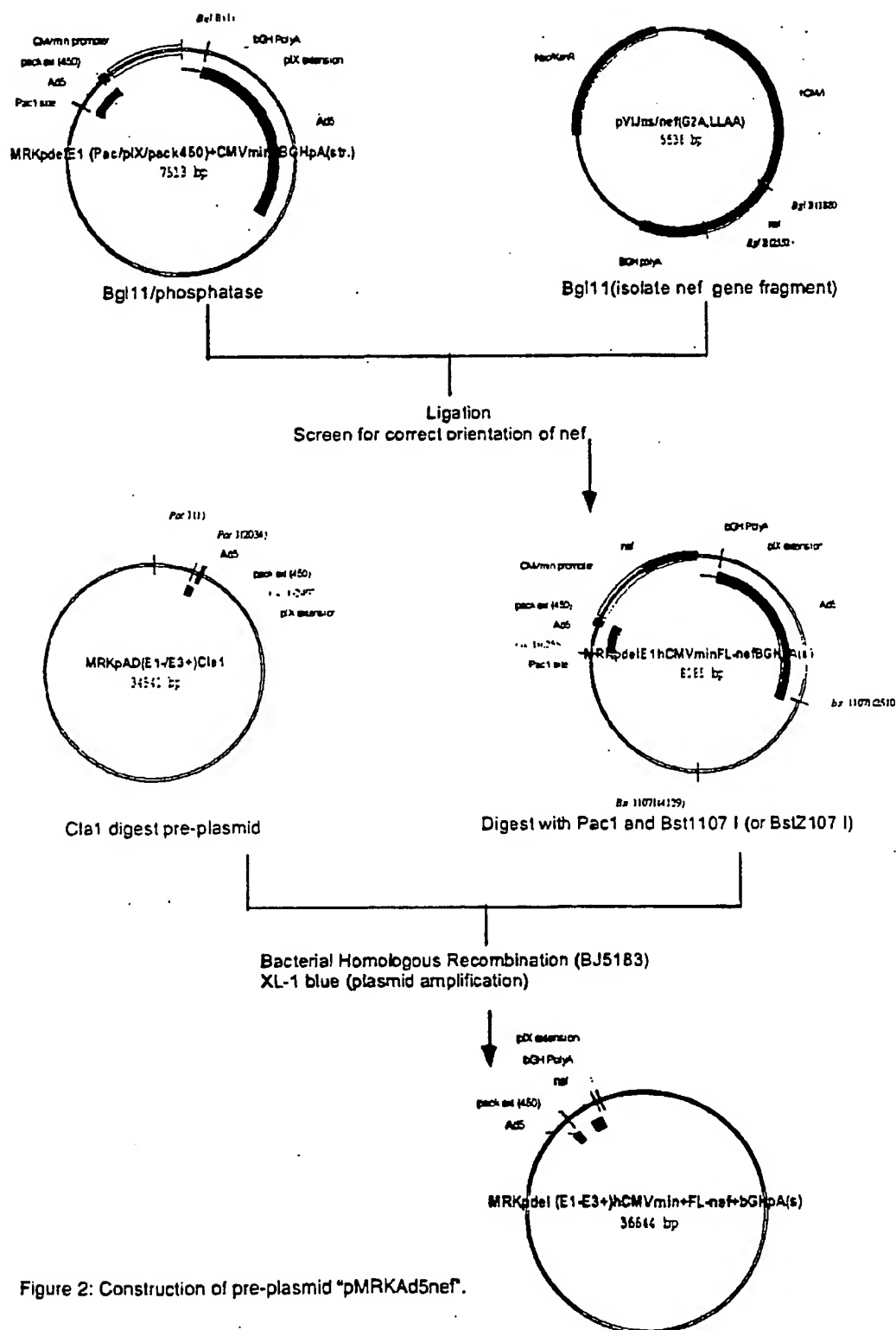
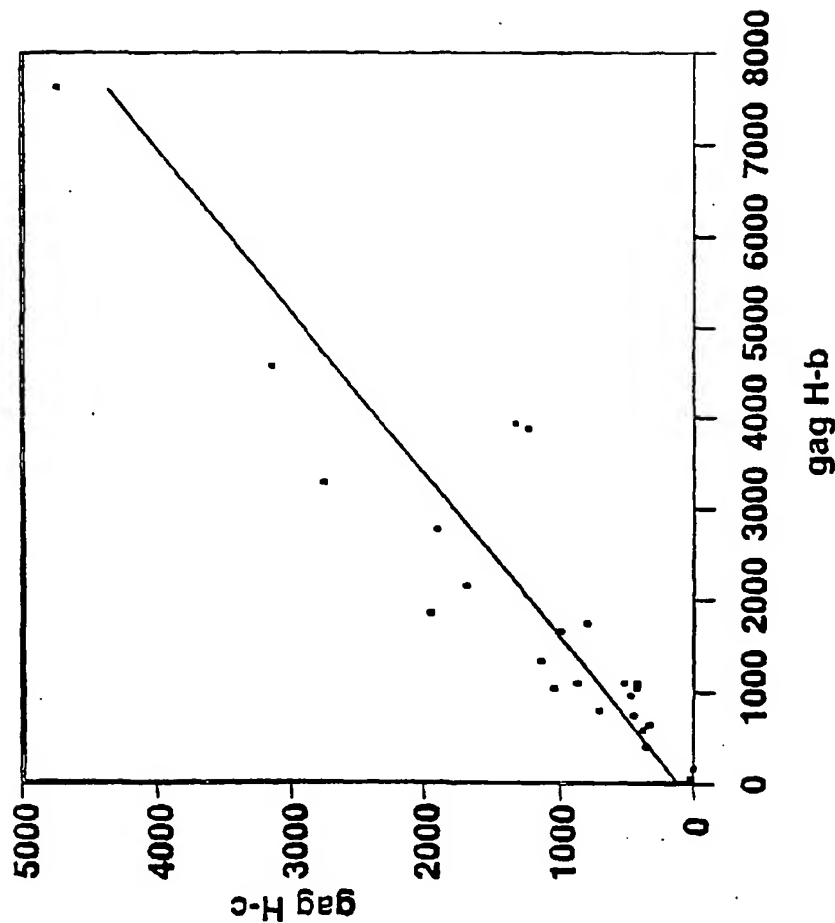


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



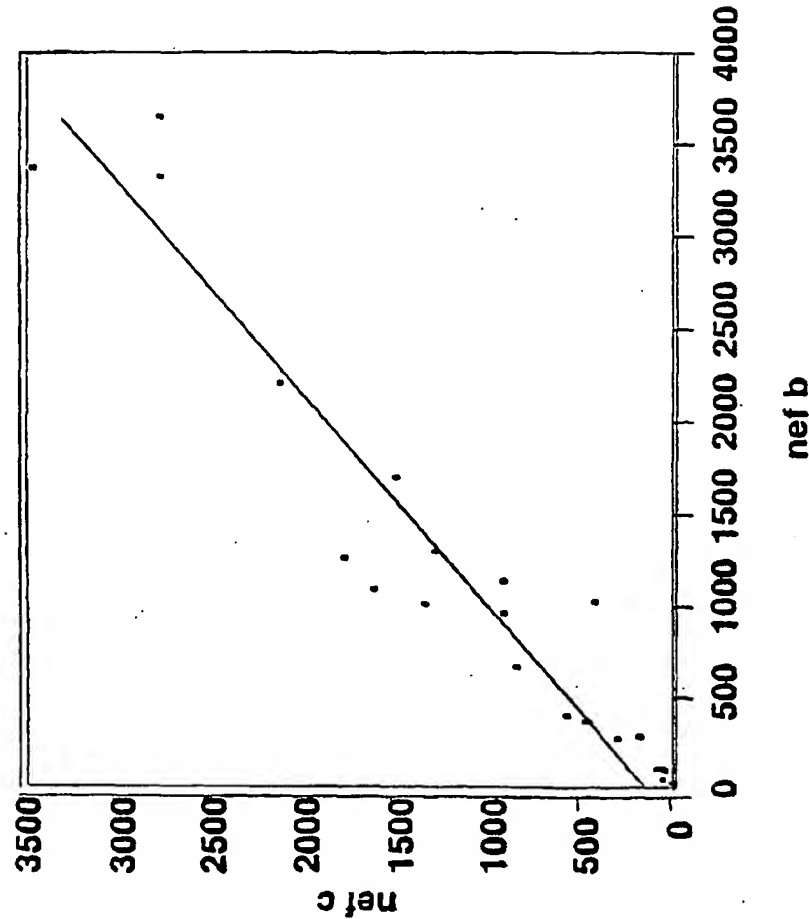
Linear Fit

$$\text{gag H-c} = 111.603 + 0.55866 \text{ gag H-b}$$

Summary of Fit

RSquare	0.816775
RSquare Adj	0.80914
Root Mean Square Error	474.9639
Mean of Response	1158.115
Observations (or Sum Wgts)	26

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



$$\text{nef c} = 131.132 + 0.8646 \text{ nef b}$$

Summary of Fit				
RSquare				0.91685
RSquare Adj				0.91289
Root Mean Square Error				289.7718
Mean of Response				1096.435
Observations (or Sum Wgts)				23

FIGURE 25

MRKAd5pol MER1062
(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCCTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCCTAC ACCGTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAAC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCACAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT TACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

Figure 24A

901 TCGCTATTAC C GGTGATG CGGTTTGGC AGTACATCAA TGGGCG EA
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

 951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 ATCGCCAAAC TGAGTGCCCC TAAAGGTCA GAGGTGGGGT AACTGCAGTT

 1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

 1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 TGTGAGGCG GGGTAACGTC GTTACCCGC CATCCGCACA TGCCACCCTC

 1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
 CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

 1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
 GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

 1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
 AGCGCCCGGC CTTGCCACG TAACCTTGCG CTAAGGGGC ACGGTTCTCA

 1251 GAGATCTACC ATGGCCCCCA TCTCCCCAT TGAGACTGTG CCTGTGAAGC
 CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

 1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG
 ACTTCGGACC GTACCTACCG GGGTTCCTACT TCGTCACCGG GGACTGACTC

 1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG
 CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGAATCTACC TCTTCCTCCC

 1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG
 GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

 1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
 GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

 1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC
 CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

 1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG
 GGTGGGGCGA CCGGACTTCT TCTTCTTCAG AACTGACAC GACCGACACC

 1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT
 CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTTATGTGA

 1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA
 CGGAAGTGGT AGGGGAGGTA GTTGTACTC TGGGGACCGT AGTCCATGGT

 1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT
 CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

 1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
 GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CTTCTGTCTT GGGACTGTAA

 1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT
 CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26B

1851 TGGGCAGCAC A CCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTG T
 ACCCGTCGTG TCCTGGTTCT AACTCCTCGA CTCCGTCGTG GACGACTCA
 1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG
 CCCCGGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC
 1951 TGGATGGGCT ATGAGCTGCA CCCCACAAAG TGGACTGTGC AGCCCATTTGT
 ACCTACCCGA TACTCGACGT GGGGCTGTTT ACCTGACACG TCGGGTAACA
 2001 GCTGCCTGAG AAGGACTCCT GGA CTGTGAA TGACATCCAG AAGCTGGTGG
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT
 GACACGTTCTG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG
 CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC
 2201 AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC
 TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTAGA TGGTCTTCGG
 2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCCACA
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GG GTGT
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
 GGTACTACA CTTCGTCGAC TGA CTCCGAC ACGTCTTCTA GTGGTGACTC
 2401 TCCATGTGTA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA
 AGGTAACT AGACCCGTT CTGGGGGTTT AAGTTCGACG GGTAGGTCTT
 2451 GGAGACCTGG GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG
 GACTCACCTT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC
 2551 CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC
 GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGGAAGATAC ACCGACCCCG
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG
 ACGGTTGTCC CTCTGGTTCTG ACCCGTTCCG ACCGATACAC TGGTTGTCCC
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG
 2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT
 GAGGTCCGGT AGATGGACCG GGAGGTCCTG AGACCGGACC TCCACTTGTA
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC
 AACTGACGG AGGGTCATAC GGGACCCGTA GTAGSTCCGG CTCGGACTAG

Figure 26 C

2801 AGTCTGAGTC TCTGGTG AACCAGATCA TTGAGCAGCT GATCAA G
 TCAGACTCAG ACTCGACCAC TTGGTCTAGT AACTCGTCGA CTAGTCTTC
 2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA
 CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCGTT
 2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTC
 ACTCGTCCAC CTGTTGACAC ACAGACGACC GTAGTCTTC CACGACAAGG
 2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
 ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG
 3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA
 ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT
 3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG
 CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC
 3101 GGCAGGTGGA CTGCTCCCTT GGCATCTGGC AGCTGGCCTG CACCCACCTG
 CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC
 3151 GAGGGCAAGG TGATCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA
 CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAT
 3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC
 CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG
 3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
 ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG
 3301 TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT
 AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA
 3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG
 GTTCGTCTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC
 3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG
 GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC
 3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTC TCCACAACCT
 CGACTCGTGG ACTTCTGTG ACACGTCTAC CGACACAAGT AGGTGTTGAA
 3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG
 GTTCTCCTTC CCCCCGTAGC CCCCAGTGG GCGACCCCTC TCCTAACACC
 3551 ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
 TGTAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG
 3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG
 TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCTT TGGGGGACAC
 3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC
 CTTCCCGGGA CGGTTGACG ACACCTTCCC CTTCCCCGA CACCACTAGG
 3701 AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC
 TCCTGTTGAG ACTGTAGTTC CACCACGGGT CTTCTTCCG GTTCTAGTAG

Figure 26 D

3751 AGGGACTATG CAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCATCA
 TCCCTGATAC CCGTCGTCTA CCGACCCCTA CTGACACACC GGAGGTCTGT
 3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
 CCTACTCCTG ATTTCCGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG
 3851 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
 GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACGGGA CCTTCCACGG
 3901 ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTTGTCT
 TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA
 3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
 CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC
 4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
 CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA
 4051 ATGGCCGATC GGCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
 TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTTCCAC
 4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC
 CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG
 4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTTGTGA
 TCGTCGGCGG CGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT
 4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
 CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA
 4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCTTGCCCG CAACTCTAC
 CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG
 4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT
 ATGGAACATGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA
 4351 CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
 GCGCGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG
 4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC
 AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG
 4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
 GGCCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAACTGGG
 4501 GGGAACTTAA TGTGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT
 CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTGCTCCAA
 4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA
 AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT
 4601 AAAACCAGAC TCTGTTTGA TTTGGATCAA GCAAGTGTCT TGCTGTCTTT
 TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA
 4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCG GGGACCAGCG GTCTCGGTCTG
 TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCTG CAGAGCCAGC

Figure 26E

4701 TTGAGGGTCC TGTGTATTTT TTCCAGGACG TGGTAAAGGT-GACTCTGAT
AACTCCCAGG AATAAAA AAGGTCCTGC ACCATTTCCTA CTGAGA A

4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT
CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCACCTCC ATCGTGGTGA

4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG
CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851 GAGCGCTGGG CGTGGTGCCT AAAATGTCT TTCAGTAGCA AGCTGATTGC
CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG

4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTCAC AAAGCGGTTA AGCTGGGATG
GTCCCCGTCC GGAACCCACA TTCACAAATG TTTGCCAAT TCGACCCCTAC

4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
CCACGTATGC ACCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001 GCTATGTTCC CAGCCATATC CCTCCGGGA TTCATGTTGT GCAGAACCAC
CGATACAAGG GTCGGTATAG GGAGGCCCT AAGTACAACA CGTCTGGTG

5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCTATG AGCTTAGAAG
GTCGTGTCAC ATAGGCCACG TGAACCTTT AAACAGTACA TCGAATCTTC

5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
CTTTACGCAC CTTCTGAAC CTCTCGGGA AACTGGAGG TTCTAAAAGG

5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
TACGTAAAGCA GGTATTACTA CCGTTACCCG GGTGCCGCC GCCGGACCCG

5201 GAAGATATTT CTGGGATCAC TAACGCATA GTTGTGTTCC AGGATGAGAT
CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251 CGTCATAGGC CATTTTACAA AAGCGCGGCG GGAGGGTGCC AGACTGCGGT
GCAGTATCCG GTAAAATGT TTCGCGCCCG CCTCCACGG TCTGACGCCA

5301 ATAATGGTTC CATCCGCCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT
TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351 TTCCCACGCT TTGAGTTCAG ATGGGGGAT CATGTCTACC TCGGGGGCGA
AAGGTGCGA AACTCAAGTC TACCCCTTA GTACAGATGG ACGCCCGCT

5401 TGAAGAAAAC GGTTCGCGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
ACTTCTTTTG CCAAAGGCC CATCCCTCT AGTCGACCT TCTTTCGTCC

5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCGT AAATCACACC
AAGGACTCTG CGACGCTGAA TGGCGTCGCG CACCCGGGCA TTTAGTGTGG

5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551 TGAGCAGGGG GCGCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCG CCCAGCGATA GCAGTTCTTG
GACTGGTTTA GCGGCTCTTC CCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26F

5651 CAAGGAAGCA AATTTTCA ACGGTTGAG ACCGTCCGCC GTAGGCATC
 GTTCCTTCGT TTCAAAAAGT TGCCAAACTC TGGCAGGCGG CATCCGTACG
 5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCACAG CTCGGTCACC
 AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG
 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC
 5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA
 5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG
 GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC
 5901 TGAAGGGGTG CGCTCCGGGC TGC GCGCTGG CCAGGGTGCG CTTGAGGCTG
 ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCACGC GAACTCCGAC
 5951 GTCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCTGCG CGTCGGCCAG
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC
 6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA
 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGAGA
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT
 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
 GAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGGCCCTCAT
 6151 GGCATCCGCG CGCAGGCCCG CGCAGACGGT CTCGCATTCC ACGAGCCAGG
 CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC
 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTCCTCCC ATGCTTTTTG
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC
 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GTCGGGTGAC
 TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG
 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCTCGA
 CTTTTCGGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT
 6351 GCGGTGTTCG GCGGTCTCTC TCGTATAGAA ACTCGGACCA CTCTGAGACA
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT
 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCAACC TCCCCATCGC
 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTA AGACACATGT
 CAGCAACAGG TGATCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA
 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
 GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC
 6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGTGG GGGCGCGTTC
 ACTGGCCCCAC AAGGACTTCC CCCCATATT TTCCCCACC CCCGCGCAAG

Figure 266

6601 GTCCTCACTC TCTTCCGCAT CGCTGTCTGC GAGGGCCAGG TGTGTGCTTG
 CAGGAGTGAG AAGGCGTA GCGACAGACG CTCCCGGTGC ACAACGAC
 6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCACTT
 TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTG TAACAGTCAA
 6701 TCCAAAAACG AGGAGGATTT GATATTCACT TGGCCCGCGG TGATGCCTTT
 AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA
 6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA
 CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT
 6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTGCGCGATG
 CGAACCACCG TTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC
 6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT
 CTCGCGTCCC AAACCAAAAA CAGCGCTAGC GCGCGAGGA ACCGGGCGCTA
 6901 GTTATAGCTG ACGTATTTCG GCGCAACGCA CCGCCATTTC GGAAAGACGG
 CAAATCGACG TGCATAAGCG CGCGTTGCGT GCGCGTAAGC CCTTTCTGCC
 6951 TGGTGCCTGC GTCGGGCACC AGGTGCACGC GCCAACCCTG GTTGTGCAGG
 ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC
 7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT
 CACTGTTCCA GTTGCACCA CCGATGGAGA GCGCGATCCG CGAGCAACCA
 7051 CCAGCAGAGG CCGCCGCCCT TGCAGGAGCA GAATGGCGGT AGGGGGTCTA
 GGTGCTCTCC GCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT
 7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC
 CGACGCAGAG CAGGCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTGC
 7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CTTGCAAGT CTAGCGCCTG
 TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC
 7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC
 GACGGTACGC GCCCGCCGTT CCGCGCGAG CATACCCAAC TCACCCCTG
 7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAAATGTCG
 GGGTACCGTA CCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC
 7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT
 ATTTGCATCT CCCCAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA
 7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTTC TGCGAGGGAG
 AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGCTCCCTC
 7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CCGGCTGCTC TGCTCGGAAG
 GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCGGACGAG ACGAGCCTTC
 7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG
 TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC
 7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG
 CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7551 AGGCGTAGGA GCGCAGC TTGTTGACCA GCTCGGCGGT GACCTG G
 TCCGCATCCT CAGCGCGTCG AACAACTGGT CGAGCCGCCA CTGGACGTGC
 7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG
 AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC
 7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT
 AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA
 7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
 AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA
 7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
 TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAGATG
 7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG
 CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC
 7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
 GTTTCACAG GGA CTGGTAC TGAACTCCA TGACCATAAA CTTCACTCAC
 7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA
 AGCAGCGTAG GCGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT
 7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTCCCG
 TGCGCCATAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC
 8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
 GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT
 8051 CGGTGTGTTAA TTACCTGGGC GCGGAGCACG ATCTCGTCAA AGCCGTTGAT
 GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA
 8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
 CAACACCGGG TGTTACATTT CAAGTTCTT CGCGCCCTAC GGGAACTACC
 8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
 TTCCGTTAAA AAATCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG
 8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
 GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT
 8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
 ACTCGAGGTG TCCAGTGCCC GGTAAATCGTA AACGTCCACC AGCGCTTTCC
 8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
 AGGATTTGAC CGCTGATAC CCGTAAAAAA GACCCCACTA CGTCATCTTC
 8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCC GGGCTAGGTC
 CATTGCCCCA GAACAAGGGT CGCCAGGGTA GGTTCCAAGC GCCGATCCAG
 8401 TCGCGCGGCA GTCAC TAGAG GTCATCTCC GCCGAAC TTC ATGACCAGCA
 AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT
 8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
 ACTTCCCGTG CTCGACGAAG GGTTCGCGG GTAGGTTCA TATCCAGAGA

Figure 26I

8501 ACATCGTAGG TAAAGAG ACGCTCGGTG CGAGGATGCG AGCCGA G
TGTAGCATCC ACTGTTTCTC TCGAGGCCAC GTCCTACGC TCGGCTAGCC

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTA
CTTTCATCTT CAGGGACGCT GCGCGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG
TTTGACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCTT
CAACTGGACT GCTGGCGCGT GTTCTTCTGT CTCACCTTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTCTA CTTGCGCTGC TTGTCCTGA
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTGCGAGC TTGATGACAA
GCTCGGGTTT CAGGTCTACA GCGCGCGGCC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CCGCTGATG CCATGGCGCG

9101 GCGGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG
CCGCCCCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG
ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CGAGGCCTG GCGGCGCCTC

9201 AGGGGGCAGG GGCACGTCGG CGCGCGCGC GGGCAGGAGC TGGTGCTGCG
TCCCCCGTCC CCGTGCAGCC GCGGCGCGC CCCGTCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GCGGTTGAT CTCCTGAATC
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG

9301 TGGCGCTCT GCGTGAAGAC GACGGGCCCG GTGAGCTTGA ACCTGAAAGA
ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CCGTGTCTGT GACGGCGGCC TGGCGCAAAA
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCCCGGG ACCGCGTTTT

9401 TCTCTGCAC GTCTCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9451 TGCTCGATCT C CTCTCTG GAGATCTCCG CGTCCGGCTC GCTCCA T
ACGAGCTAGA GAAGGAGGAC CTCTAGAGGC GCAGGCCGAG CGAGGTGCCA

9501 GCGGGCGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCTTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CCGGAGATTG AGCTCCACGT GCCGGGCGAA
GCCGCGCGCT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGATTGCG
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
AACTATAGGG GGTTCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGCGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
CCGCTTCAAC TTTTGTACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC
AACCTTCTGC GCGGGGCACT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAAACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACGATC ACAGTCGCAA GGTAGGCTGA
TTTGAGAGC TCTTCCGCA GATTGCTCAG TGTACGCTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG
CGTGGCACCG CCCGCCGTCG CCCGCCGCA GCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GCGGATGGT
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10401 CGACAGAAGC AATGTCTCT TGGGTCCGGC CTGCTGAATG CGCAGGCTT
 GCTGCTCTTCG TGTACAGGA ACCCAGGCCG GACGACTTAC GCGTCCCTCA
 10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC
 10501 TCTTGCATGA GCCTTTCTAC CGGCACCTCT TCTTCTCCTT CCTCTTGTC
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAA CCGGCATCCA
 10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCTT CATCGGCTGA
 CCGCGGAGAG AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT
 10651 AGCAGGGGCTA GGTGCGCGAC AACCGCTTCG GCTAATATGG CCTGCTGCAC
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC
 10751 CGCCCGTGTT GATGGTGTAA GTGCAGTTGG CCATAACGGA CCAGTTAACG
 GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GCGGTGGTCC ATGACCATAG
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCCA
 10951 GCGGGGGCTC CCGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA
 CGGCCCCGAG GCCCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC
 11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG
 11101 ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CGCGTTAGCA ACTGCGAGAT
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG
 CTGGCACGTT TTCCTCTCGG ACATTGCCCC GTGAGAAGGC ACCAGACCAC
 11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA
 CTATTTAAGC GTTCCCATAG TACCGCTGCG TGGCCCCAAG CTCGGGGCAT
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA
 AGGCCGGCAG GCGGCAC TAGTACGCAAT GGCGGGCGCA CAGCTTGGGT
 11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG
 CCACACGCTG CAGTCTGTTG CCCCTCAGG AGGAAAACCG AAGGAAGGTC

Figure 26L

11351 GCGCGGCGGC TCGCGCTA GCTTTTTTGG CCACTGGCCG CGCGCACT
CGCGCCGCCG ACACGCGGAT CGAAAAACC GGTGACCGGC GCGCGTCCA

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATTA AGTGGCTCGC TCCCTGTAGC
TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCGC GTTCGAGTCT
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCGCGGGC CAAGCTCAGA

11501 CGGACCGGCC GGAATGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG
GCCTGGCCCG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCGGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTGTGCTT
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAACGAA

11601 TTCCAGATG CATCCGGTGC TCGGCGAGAT GCGCCCCCTT CCTCAGCAGC
AAGGCTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTGC

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCCT CCCTCCTCCT
CCGTCTCTGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG
AATGCTTGGG GCGCGCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GCGCGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT
CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG
AGGTGCGTCC CGCGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCGG ACCTGGTAAC CGCATACGAG CAGACGGTGA
GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT
TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT
CACCAGCGCG TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT
TTCGCGCGAC CTCGTTTGGG GTTTATCGTT CGCGAGTAC CGCGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTGAG GGATGCGCTG
AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12301 CTAAACATAG T G C C C C G A G G G C C G C T G G C T G C T C G A T T T G A T A A T
 G A T T T G T A T C A T C T C G G G C T C C C G G C G A C C G A C G A G C T A A A C T A T T T G T A

12351 C C T G C A G A G C A T A G T G G T G C A G G A G C G C A G C T T G A G C C T G C T G A C A A G G
 G G A C G T C T C G T A T C A C C A C G T C C T C G C G T C G A A C T C G G A C C G A C T G T T C C

12401 T G G C C G C C A T C A A C T A T T C C A T G C T T A G C C T G G G C A A G T T T T A C G C C C G C
 A C C G G C G G T A G T T G A T A A G G T A C G A A T C G G A C C C G T T C A A A A T G C G G G C G

12451 A A G A T A T A C C A T A C C C C T T A C G T T C C C A T A G A C A A G G A G G T A A A G A T C G A
 T T C T A T A T G G T A T G G G G A A T G C A A G G G T A T C T G T T C C T C C A T T T C T A G C T

12501 G G G G T T C T A C A T G C G C A T G G C G C T G A A G G T G C T T A C C T T G A G C G A C A C C
 C C C C A A G A T G T A C G C G T A C C G C G A C T T C C A C G A A T G G A A C T C G C T G C T G G

12551 T G G G C G T T T A T C G C A A C G A G C G C A T C C A C A A G C C G T G A G C G T G A G C C G G
 A C C C G C A A A T A G C G T T G C T C G C G T A G G T G T T C C G G C A C T C G C A C T C G G C C

12601 C G G C G C G A G C T C A G C G A C C G C G A G C T G A T G C A C A G C C T G C A A A G G G C C C T
 G C C G C G C T C G A G T C G C T G G C G C T C G A C T A C G T G T C G G A C G T T T C C G G G A

12651 G G C T G G C A C G G G C A G C G C G A T A G A G A G G C C G A G T C C T A C T T T G A C G C G G
 C C G A C C G T G C C C G T C G C C G C T A T C T C T C C G G C T C A G G A T G A A A C T G C G C C

12701 G C G C T G A C C T G C G C T G G G C C C A A G C C G A C G C G C C C T G G A G G C A G C T G G G
 C G C G A C T G G A C G C G A C C C G G G G T T C G G C T G C G C G G A C C T C C G T C G A C C C

12751 G C C G G A C C T G G C T G G C G G T G G C A C C C G C G C G C G T G G C A A C G T C G G C G G
 C G G C C T G G A C C G A C C G C C A C C G T G G G C G C G C G A C C G T G C A G C C G C C

12801 C G T G G A G G A A T A T G A C G A G G A C G A T G A G T A C G A G C C A G A G A C G G C G A G T
 G C A C C T C C T T A T A C T G C T C C T G C T A C T C A T G C T C G G T C T C T G C C C G C T C A

12851 A C T A A G C G G T G A T G T T T C T G A T C A G A T G A T G C A A G A C G C A A C G G A C C C G G
 T G A T T C G C C A C T A C A A A G A C T A G T C T A C T A C G T T C T G C G T T G C C T G G G C C

12901 C G G T G C G G G C G G C G C T G C A G A C C A G C C G T C C G G C C T T A A C T C C A C G G A C
 G C C A C G C C C G C C G C G A C G T C T C G G T C G G C A G C C G G A A T T G A G T G C C T G

12951 G A C T G G C G C C A G G T C A T G G A C C G A T C A T G T C G C T G A C T G C G C G A A T C C
 C T G A C C G C G G T C C A G T A C C T G G C G T A G T A C A G C G A C T G A C G C G C G T A G G

13001 T G A C G C G T T C C G G C A G C A G C G C A G G C C A A C C G G C T C T C C G C A A T T C T G G
 A C T G C G C A A G G C C G T C G T C G C G T T G C G T C C G G T T G C C G A G A G G C G T T A A G A C C

13051 A A G C G G T G G T C C C G G C G C G C G C A A C C C C A C G C A C G A G A A G G T G C T G G C G
 T T C G C C A C C A G G C C G C G C G T T T G G G G T G C G T G C T C T T C C A C G A C C G C

13101 A T C G T A A A C G C G C T G G C C G A A A C A G G G C C A T C C G G C C C G A C G A G G C C G G
 T A G C A T T T G C G C G A C C G C T T T T G T C C C G G T A G G C C G G G C T G C T C C G G C C

13151 C C T G G T C T A C G A C G C G C T G C T T C A G C G C G T G G C T C G T T A C A A C A G C G G C A
 G G A C C A G A T G C T G C G C G A C G A A G T C G C G C A C C G A G C A A T G T T G T C G C C G T

13201 A C G T G C A G A C C A A C C T G G A C C G G C T G G T G G G G A T G T G C G C G A G G C C G T G
 T G C A C G T C T G G T T G G A C C T G C C G A C C A C C T A C A C G C G C T C C G G C A C

Figure 26 N

13251 GCGCAGCGTG A GCGCGCA GCAGCAGGGC AACCTGGGCT CCATGGGTC
CGCGTCGCAC TCGCGCGCGT CGTCGTCCCG TTGGACCCGA GGTACCAACG

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG
TGATTTGCCG AAGGACTCAT GTGTCGGGCG GTTGACAGGC GCCCCTGTCC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA
TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG
GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAATTGTC
ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG

13501 AGGGGCTGTG GGGGGTGCAG GCTCCACAG GCGACCGCGC GACCGTGTCT
TCCCCGACAC CCCCCACGCC CGAGGGTGTG CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT
TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA
GTGCCGTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC
GTGACATGGC GCTCCGGTAT CCACTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG
GTCTCTAAT GTTACAGTC GCGCGCGGAC CCCGTCTCC TGTGCCCCGT

13751 CCTGGAGGCA ACCCTAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC
GGACCTCCGT TGGGATTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG
GGAGCAACGT GTCAAAATTG TCGCTCTCC TCGCGTAAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGTAA CGCCCAGCGT
GTCGTCTCGC ACTCGGAATT GGAATACGCG CTGCCCCATT GCGGGTCGCA

13901 GCGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA
CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCAATG CGCGGCCGCC
TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTAC CAATGCCATC TTGAACCCGC ACTGGCTACC
CACTTGGGCG TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTGGA GGTGCCCCGAG GGTAAACGATG
CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101 GATTCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG
CTAAGGAGAC CTGCTGTAT CTGCTGTGCG ACAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA
TGGGACGATC TCAACGTTGT CGCGCTCGTC CGTCTCCGC GCGACGCTTT

Figure 260

14201 GGAAAGCTTC CCGAGGCCAA GCAGCTTGTC CGATCTAGGC GCTGCGGCTC
 CCTTTCGAAG GCTCCGGTT CGTCGAACAG GCTAGATCCG CGACGCTG
 14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC
 GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG
 14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA
 TCGTGAGCGT GGTGGGCGGG CCGGACGAC CCGCTCCTCC TCATGGATTT
 14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTC
 GTTGAGCGAC GACGTCGGCG TCAGCGCTTTT TTTGGACGGA GGCCGTAAAG
 14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG
 GGTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC
 14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG
 ATGCGCGTCC TCGTGTCCTT GCACGCTCCG GCGCGGGCGG GGTGGGCAGC
 14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG
 AGTTTCCGTG CTGGCAGTCG CCCAGACCA CACCCTCCTG CTACTGAGCC
 14551 CAGACGACAG CAGCGTCTG GATTGGSAG GGAGTGGCAA CCCGTTTGGC
 GTCTGCTGTC GTGCGAGGAC CTAAACCTC CCTCACCGTT GGGCAAACGC
 14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA
 GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT
 14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTCTT
 ACGTTTATTT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA
 14701 GTATTCCCTT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCCTCCT
 CATAAGGGGA ATCATAAGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA
 14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG
 GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCAACCGC GCCGCGACCC
 14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC
 AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GCGGCCATGG
 14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC
 ACGCCGGATG GCGCCCTCT TTGTCGTAGG CAATGAGACT CAACCGTGGG
 14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT
 GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCTTACA
 14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA
 CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAGAC TGGTGCCAGT
 15001 TTCAAACAA TGACTACAGC CCGGGGAGG CAAGCACACA GACCATCAAT
 AAGTTTGTG ACTGATGTG GCGCCCTCC GTTCGTGTGT CTGGTAGTTA
 15051 CTTGACGACC GGTGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC
 GAAGTGTGTT CCAGCGTGAC CCGCCGCTG GACTTTTGGT AGGACGTATG
 15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC
 GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATCCGCG

Figure 26 P

15151 GGGTGATGGT GCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTA
CCCCTACCA CAGCGCGAAC GGATGATTCC TGTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCAG GCAACTACT CCGAGACCAT
ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTGTATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG
CTGGTATCTG GAATACTTGT TCGCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC
CGTCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCTTA

15451 GCGGGGTGGA CTTCAACCCAC AGCCGCTGA GCAACTTGT GGGCATCCGC
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA
TTCCCGCTTG GGAAGTCCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAC ATTCCCGCAC TGTGGATGT GGAAGCTTAC CAGGCGAGCT
CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GCGGGGGGTG GCGCAGGCGG CAGCAACAGC
ACTTCTACT GTGGCTGTG CCGCCCCAC CGCGTCCGC GTCGTGTGCG

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA
TCACCGTCCG CGCGCTTCT CTTGAGGTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGCGAC ACCTTTGCCA
CGGCCACCTC CTGTACTGTC TAGTACGGTA AGCGCCGCTG TGGAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC
GTGCCCCACT CCTCTTCGCG CCACTCCGGC TTCGTGCGCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTGAGAGA CCTCAGAAGA AACCAGGTGAT
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA
GTTTGGGGAC TGCTCCTGT CGTCTTTGC GTCAATGTTG GATTATTCGT

15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAATAC
TACTGTCTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GCGCACCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA
CCGCTGGGAG TCTGGCCTTA GCGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 A

16101 GTGGGCGCGC A TGGTTGCC CGTGCCTCC AAGAGCTTCT ACAACG TCA
CACCCGCGGC TACAACGG GCACGTGAGG TTCTCGAAGA TGGTGC GT

16151 GGCCGTCTAC TCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT
CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201 TCAATCGCTT TCCCAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC
AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251 ATCACCACCG TCAGTGAAAA CGTTCTTGCT CTCACAGATC ACGGGACGCT
TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG
TGGCGACGCG TTGTCGTAGC CTCTCAGGT CGCTCACTGG TAATGACTGC

16351 CCAGACGCGC CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG
GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401 CCGCGCGTCC TATCGAGCCG CACTTTTGA GCAAGCATGT CCATCCTTAT
GGCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCGTACA GGTAGGAATA

16451 ATCGCCACAG AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT
TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA

16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG
AACCGCCCGG GTTCTTCGCG AGGCTGGTGG TGGGTACGCG GCACGCGCCC

16551 CACTACGCGC CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC
GTGATGGCGC GCGGGACCCC GCGCGTGTTC GCGCCGGCGT GACCCGCGTG

16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA
GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCTCCGC GCGTTGATGT

16651 CGCCACGCC GCCACAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG
GCGGGTGGCG GGTGGTACAC AGGTGTCACC TCGCCCGTA AGTCTGGCAC

16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT
CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751 AGCACGTGCG CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG
TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGCGGGGT GCGCGCCGCC

16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGG GGCATGCGG
GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCC CCGGTACGCC

16851 GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG
CGGCGAGCTT CCGACCGCGC CCCATAACAG TGACACGGGG GTCCAGGTG

16901 GCGACGAGCG GCCGCCGCG CAGCCGCGGC CATTAGTGCT ATGACTCAGG
CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951 GTCGACGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC
CAGCGTCCCC GTTGACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG

17001 GTGCCCCGTC GCACCCGCC CCCGCGCAAC TAGATTGCAA GAAAAACTA
CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTGTAT

Figure 26 R

17051 CTTAGACTCG TTTGTTGTA TGTATCCAGC GCGGGCGGCG CCGAACTG
 GAATCTGAGC ATGACAACAT ACATAGGTCT CCGCCGCCGC GCGTTGCTTC
 17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG
 GATACAGGTT CCGGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC
 17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA
 CTCTAGATAC CCGGGGGCTT CTTCTTCTCT GTCCTAATGT TCGGGGCTTT
 17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG
 CGATTTCCGC CAGTTTTTCT TTTCTTTCT ACTACTACTA CTGAACTGC
 17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG
 TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC
 17301 AAAGGTCGAC GCGTAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT
 TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA
 17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG
 ATCGGGGCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC
 17401 TGTCAGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG
 ACATGCCGCT GCTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCTC
 17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA
 AAACGGATGC CTTTCGCCGT ATTCTGTAC GACCGCAACG GCGACCTGCT
 17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC
 CCCGTTGGGT TGTGGATCGG ATTCGGGCA TTGTGACGTC GTCCACGACG
 17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT
 GGCGCGAACG TGGCAGGCTT CTTTCGCGC CGGATTTGCG GCTCAGACCA
 17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA
 CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT
 17651 AGATGTCTTG GAAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC
 TCTACAGAAC CTTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG
 17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCCG GACTGGGCGT GCAGACCGTG
 CGCACGCCCG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC
 17751 GACGTTGAGA TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA
 CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GCGGTGTCT
 17801 GGGCATGGAG ACACAAACGT CCCCGGTTGC CTCAGCGGTG GCGGATGCCG
 CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCTACGGC
 17851 CCGTGCAGGC GGTGCTGCG GCGCGCTCCA AGACCTCTAC GGAGGTGCAA
 GCCACGTCCG CCAGCGACGC CCGCGCAGGT TCTGGAGATG CCTCCACGTT
 17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCGCGGCC CGCGCCGTTT
 TGCCTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCCGCG GCGCGGCAAG
 17951 GAGGAAGTAC GCGCGCGCCA GCGCGTACT GCGCGAATAT GCCCTACATC
 CTCCTTCATG CCGCGCGCGT CCGCGGATGA CCGGCTTATA CCGGATGTAG

Figure 265

18001 CTTCCATTGC GCTACCCCC GGCTATCGTG GCTACACCTA CCGGCCGAGA
 GAAGGTAACG ATGGGGG CCGATAGCAC CGATGTGGAT GGCGGG T
 18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG
 TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGCGCGCGGC
 18101 TCGCCGTGCG CAGCCCCGTC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC
 AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG
 18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCAGC
 CGCTTCCTCC GTCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTGC
 18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCTCACCTG
 TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC
 18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA
 GCGCGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT
 18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGGCACCAC
 CCCGTACCG GCCGGTGCCG GACTGCCCCG CGTACGCAGC ACGGCTGGTG
 18351 CGCGCGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCT
 GCCCGCCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA
 18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT
 GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA
 18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG
 GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC
 18501 AAAAAATCAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC
 TTTTATAGTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG
 18551 TATTTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC
 ATAAAAATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG
 18601 GGCTCGCGCC CGTTCATGGG AACTGGCAA GATATCGGCA CCAGCAATAT
 CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTGTTATA
 18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT
 CTCGCCACCG CGGAAGTCGA CCCCAGCGCA CACCTCGCCG TAATTTTAA
 18701 TCGGTTCAC CGTTAAGAAC TATGGCAGGA AGGCCTGGAA CAGCAGCACA
 AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT
 18751 GGCCAGATGC TGAGGGATAA GTTGAAGAG CAAAATTTCC AACAAAAGGT
 CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTCCA
 18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC
 CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTTGG
 18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA
 TCCGTCACGT TTTATTCTAA TTGTCATTG AACTAGGGGC GGGAGGGCAT
 18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GCGGTGGCGA
 CTCCTCGGAG GTGGCCGGCA CCTCTGTAC AGAGGTCTCC CCGCACCGCT

Figure 26T

18951 AAAGCGTCCG C●●CCGACA GGAAGAAAC TCTGGTGACG CAAATAC●●G
 TTTCGCAGGC G●●GGGCTGT CCCTTCTTTG AGACCACTGC GTTTATC●●G

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT
 TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC
 GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG
 CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC
 CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA
 CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTACG CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC
 TTCGTGTGAC TTGTGCTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC
 CTGTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTGCGCCG CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA
 TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCAGCGGGCG GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC
 ACCGATGGGG AAGCTACTAC GGCGTCACCA GAATGTACGT GTAGAGCCCC

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG
 GTCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG
 GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG
 GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT
 AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT
 GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT
 AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCACATGCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA
 CCGTGACGGA TGTGCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG
 TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAPCGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAACTCAC
 TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u

19901 GTATTGGGC A GCCTTA TTCTGGTATA AATATTACAA AGGAGG T
CATAAACCCG TCCGCGGAAT AAGACCATAT TTATAATGTT TCCTCCCTA

19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTT
AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG

20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT
TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTTG TCTTTAATTA

20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA
GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT

20101 CGGTTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG
GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAC

20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC
ATTTTCGTTGT TTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT
AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTC

20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT
CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301 CTTACATGCC CACTATTAAG GAAGGTAAC CACGAGAACT AATGGGCCAA
GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT

20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT
GTTAGATACG GGTGTCCGG ATTAATGTAA CGAAAATCCC TGTTAAATA

20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC
ACCAGATTAC ATAATGTTGT CGTGCCCAT ATACCCACAA GACCGCCCGG

20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG
TTCTAGCGT CAACTTACGA CAACATCTAA ACGTCTCTGTC TTTGTGTCTC

20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTAATT
GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA
AAGATACACC TTAGTCCGAC AACTGTGAT ACTAGGTCTA CAATCTTAAT

20601 TTGAAAATCA TGGAAGTAA GATGAACTTC CAAATTACTG CTTTCCACTG
AACTTTTAGT ACCTTGACTT CTAATTGAAG GTTTAATGAC GAAAGGTGAC

20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAC CTAAACAGG
CCTCCACACT AATTATGCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG
AGTCCTTTTA CCTACCCTTT TTCTACGATG TCTTAAAAGT CTATTTTAC

20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC
TTTATCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG

20801 CTGTGGAGAA ATTTCTGTG CTCCAACATA GCGCTGTATT TGCCCGACAA
GACACCTCTT TAAAGGACAT GAGGTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 v

20851 GCTAAAGTAC AGCTCTTCCA ACGTAAAAAT TTCTGATAAC CCAAACTTTT
 CGATTTTCATG TCGGAAGGT TGCATTTTTA AAGACTATTG GGTTCGCTAA
 20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC
 TGCTGATGTA CTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG
 20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC
 TAATTGGAAC CTCGTGCGAC CAGGGAACTG ATATACCTGT TGCAGTTGGG
 21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG
 TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC
 21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT
 CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA
 21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGA
 CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT
 21151 CTTCAGGAAG GATGTTAACA TGTTTCTGCA GAGCTCCCTA GGAAATGACC
 GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG
 21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC
 ATTCCCAACT GCCTCGGTG TAATTCAAAC TATCGTAAAC GGAAATGCGG
 21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT
 TGAAGAAGG GGTACCGGT GTGTGGCGG AGGTGCGAAC TCCGGTACGA
 21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA
 ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT
 21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC
 TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTCACGG GTATAGGTAG
 21401 CCCTCCCGCA ACTGGGCGGC TTCCGCGGC TGGGCCTTCA CGCGCCTTAA
 GGGAGGGCGT TGACCCGCGG AAAGGCGCGG ACCCGGAAGT GCGCGGAATT
 21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACCTT
 CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA
 21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC
 TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG
 21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA
 AAATCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT
 21601 TGACCGCTG CTTACCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG
 ACTGGCGGAC GAATGGGGGT TGCTCAAACT TTAATTGCGG AGTCAACTGC
 21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCTG
 CCTCCCAAT GTTGAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC
 21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC
 CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG
 21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA
 TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTGCGGT

Figure 26 W

21801 TGAGCCGTCA GCTGGTGGAT GATACTAAAT ACAAGGAOTALECAACACCTG
ACTCGGCAGT CACCTA CTATGATTTA TGTTCCTGAT GGTGTAC

21851 GGCATCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC
CCGTAGGATG TGGTTGTGTT GTTGAACCT AAACAACCGA TGGAAACGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA
GTGGTACGCG CTTCTGTCTC GGATGGGACG ATTGAAGGGG ATAGGCCAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTCAA AGAAACGCTA

22001 CGCACCCCTTT GCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACCTCC GCCACGCGC
TGAGTGTCTG GACCCGGTTT TGAAGAGAT GCGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCAC CCTTCTTTAT
ATCTGTACTG AAACTCCAC CTAGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTGTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACGCGG
CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGCTG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCACGCGC CTTCTCGGCC GGCAACGCCA
GCAGTAGCTT TGGCACATGG ACGCGTGGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA
GTGTATTTC TTCGTTTCGT GTAGTTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGTTG TGGGCCATAT
CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351 TTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA
AAAAACCCGT GGATACTGTT CCGGAAAGGT CCGAAACAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCCG TCGCGAGACT GGGGCGGTAC
CGAGCGGACG CCGTATCAGT TATGCCGCC AGCGCTCTGA CCCCCGATG

22451 ACTGGATGGC CTTTGCCCTG AACC CGCACT CAAAAACATG CTACCTCTTT
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA
CTCGGGAAAC CGAAAAGACT GGTGCTGAG TTCGTCCAA TGGTCAAAT

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAGAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTG CCAACTGGCC
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC
GGTTTGAGGG TACCTAGTGT TGGGGTGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22751 CCAACTCCAT GCTCAACAGT CCCCAGGTAC AGCCACAGGT GGTTCAGGTA
 22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCCGCAG
 22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGCAC TTGAAAAACA
 22901 TGTAATAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT
 22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT
 23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG
 23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC
 23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC
 23151 CAACGCGTTT AGCAGGTGCG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC
 23201 CTCCGCCCTG CCGCGCGGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC
 23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTGCGAGAT
 23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT
 23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCAGGCTT TGAGTTGCAC
 23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG
 23451 ATACAGCGCC TGCATAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT
 23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AACTGATTG
 23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTGGAGAT
 23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG
 23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTCA

Figure 26Y

23701 ATCACGTGCT CATTATTAT CATAATGCTT CCGTGTAGAC ACTTAATTC
 TAGTGCACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG
 23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT
 CGGAAGCTAG AGTCGCGTCG CCACGTGGT GTTGC GCGTC GGGCACCCGA
 23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG
 GCACTACGAA CATCCAGTGG AGACGTTGC TGACGTCCAT GCGGACGTCC
 23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG
 TTAGCGGGGT AGTAGCAGTG TTCCAGAAC AACGACCACT TCCAGTCGAC
 23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTCATACG GCCGCCAGAG
 GTGGGCGCC ACCAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC
 23951 CTCCACTTG GTCAGGCAGT AGTTGAAGT TCGCCTTAG ATCGTTATCC
 GAAGGTGAAC CAGTCCGTCA TCAAACCTCA AGCGGAAATC TAGCAATAGG
 24001 ACGTGGTACT TGTCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCA
 TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT
 24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCCTA ATTCACCTT
 GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTGAAA
 24101 CCGCTTCGCT GGGCTCTTCC TCTTCTCTT GCGTCCGCAT ACCACGCGCC
 GCGGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGC GCGG
 24151 ACTGGGTGCT CTTCATTCAG CCGCGCACT GTGCGCTTAC CTCCTTTGCC
 TGACCCAGCA GAAGTAAGTC GCGGCGTGA CACGCGAATG GAGGAAACGG
 24201 ATGCTTGATT AGCACCGGTG GGTGCTGAA ACCCACCATT TGATCGCCA
 TACGAACTAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT
 24251 CATCTTCTCT TTCTTCTCTG CTGTCCACGA TTACCTCTGG TGATGGCGGG
 GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC
 24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC
 GCGAGCCCGA ACCCTCTTCC CCGGAAGAAA AAGAAGAACC CGCGTTACCG
 24351 CAAATCCGCC GCCGAGGTG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA
 GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT
 24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCTC
 CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TCGGCGGAG
 24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGCC GCGGCGACG GGGACGGGA
 TAGGCGAAAA AACCCCGCG GCGCCCTCCG CCGCCGCTGC CCCTGCCCTT
 24501 CGACACGTCC TCCATGGTTG GGGGACGTG CGCCGCACCG CGTCCGCGCT
 GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA
 24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC
 GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG
 24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC
 ATATCCGTCT TTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

Figure 262

24651 CGCCCCCTCT GTCGCCA CCACCGCCTC CACCGATGCC GCUAAC TC
 GCGGGGGAGA CTC AAGCGGT GGTGGCGGAG GTGGCTACGG CGGTTGCGCG
 24701 CTACCACCTT CCCCGTCGAG GCACCCCGCG TTGAGGAGGA GGAAGTGATT
 GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA
 24751 ATCGAGCAGG ACCCAGGTTT TGTAAAGCGAA GACGACGAGG ACCGCTCAGT
 TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA
 24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG
 TGGTTGTCTC CTATTTTTCG TTCTGGTCTT GTTGCCTCTC CGTTTGCTCC
 24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA
 TTGTTACGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT
 24901 GACGACGTGC TGTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA
 CTGCTGCAGG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT
 24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCTT CGCCATAGCG GATGTCAGCC
 GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG
 25001 TTGCCTACGA ACGCCACCTA TTCTCACCGC GCGTACCCCC CAAACGCCAA
 AACGGATGCT TCGGCTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT
 25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGGTATT
 CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA
 25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACGCA
 ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAG GTTTTGACGT
 25151 AGATACCCCT ATCCTGCCGT GCCAACCACA GCCGAGCGGA CAAGCAGCTG
 TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC
 25201 GCCTTGCGGC AGGGCGCTGT CATACCTGAT ATCGCCTCGC TCAACGAAGT
 CCGAACGCCG TCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA
 25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG
 CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC
 25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACCTCTGG AGTGTTGGTG
 GAGACGTTGT CCTTTTGTG CTTTTACTTT CAGTGAGACC TCACAACCAC
 25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA
 CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT
 25401 GGTCACCCAC TTTGCCTACC CGGCACCTAA CCTACCCCCC AAGGTCATGA
 CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT
 25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG
 CGTGTCAGTA CTACTCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC
 25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA
 CTACGTTTAA ACGTTCTTGT TTGTCTCTC CCGGATGGGC GTCAACCGCT
 25551 CGAGCAGCTA CGCGGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG
 GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26 AA

25601 AGCGACGCAA AATGATG GCCGCACTGC TCGTTACCGT GGAGCTAG
 TCGCTGCGTT TGATTACTAC CCGCGTCACG AGCAATGGCA CCTCGAACTC
 25651 TGCATGCAGC GGTTCCTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA
 ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT
 25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA
 TTGTAACGTG ATGTGGAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT
 25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC
 AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAACGTG
 25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC
 CTTTGGGCGG AACCCTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG
 25851 GCGCGCGGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT
 CGCGGCGCTG ATGCAGGCGC TGACGCAAT GAATAAAGAT ACGATGTGGA
 25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC
 CCGTCTGCGG GTACCCGCAA ACCGTCTGCA CGAACCTCCT CACGTTGGAG
 25951 AAGGAGCTGC AGAACTGCT AAAGCAAAC TTGAAGGACC TATGGACGGC
 TTCTCGACG TCTTTGACGA TTTGTTTTG AACTTCTG ATACCTGCCG
 26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGGACATC ATTTTCCCCG
 GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCTGTAG TAAAAGGGG
 26051 AACGCCGTGCT TAAAACCTG CAACAGGCTC TGCCAGACTT CACCACTCAA
 TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT
 26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT
 TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCA GTCTTAGAA
 26151 GCGCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC
 CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG
 26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC
 CGCTTACGGG AGGCGGCGAA ACCCGGTGA CGATGGAAGA CGTCGATCGG
 26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG
 TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC
 26301 TCTACTGGAG TGTCACGTG GCTGCAACCT ATGCACCCCG CACCGCTCCC
 AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG
 26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT
 ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGA
 26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTGAA
 CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCGAG GCCCAACTT
 26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAA TTTGTACCTG
 TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC
 26501 AGGACTACCA CGCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG
 TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG ACCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCCTGG
 GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAGAACC
 26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG
 GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC
 26651 GACGGGGGGT TTACTTGGAC CCCCAGTCCG GCGAGGAGCT CAACCCAATC
 CTGCCCCCA AATGAACCTG GGGGTCAGGC CGTCCTCGA GTTGGGTTAG
 26701 CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA
 GGGGCGGGC GCGTCGGGAT AGTCGTCGTC GGCGCCCGG AACGAAGGCT
 26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG
 CCTACCGTGG GTTTTCTTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC
 26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA
 CTCTTATGA CCTGTCTAGT CCGTCTCCTC CAAAACCTGC TCCTCTCCT
 26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCTG
 CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC
 26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CGGTCGCAAT CCCCTCGCCG
 TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC
 26951 GCGCCCCAGA AATCGGCAAC CGGTTCAGC ATGGCTACAA CCTCCGCTCC
 CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGCGGAGG
 27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA
 AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCTGT
 27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA
 GGTGACCTTG GTCCCGGCCA TTCAGGTTCTG TCGGCGGCGG CAATCGGGTT
 27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC
 CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG GTTCTTGGC
 27151 CATAGTTGCT TGCITGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCCGC
 GTATCAACGA ACGAACGTTT TGACACCCC GTTGTAGAGG AAGCGGGCGG
 27201 GCTTCTTCT CTACCATCAC GCGGTGGCCT TCCCCGTAA CATCCTGCAT
 CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA
 27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA
 ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT
 27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA
 GTCGTCGCCG GTGTGTCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT
 27351 AAGCCCAAGA AATCCACAGC GCGGCGAGCA GCAGGAGGAG GAGCGCTGCG
 TTCGGGTTCT TTAGGTGTCT CCGCCGTCTG CTCTCTCTC CTCGCGACGC
 27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT
 AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GTCGAATCT TTGTCCTAAA
 27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG
 AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTCTC

Figure 26 AC

27501 CTGAAAATAA A CAGGTC TCTGCGATCC CTCACCCGCA GCTGCC TA
 GACTTTTATT TTTGTCCAG AGACGCTAGG GAGTGGGCGT CGACGGALAT
 27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC
 AGTGTTTTTCG CTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG
 27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA
 27651 TCTCAAAATT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC
 AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG
 27701 GCCAGCACCT GTTGTACGCG CCATTATGAG CAAGGAAATT CCCACGCCCT
 CGGTCTGTGA CAACAGTCGC GGTAACTCTC GTTCCTTTAA GGGTGCGGGA
 27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA
 TGTACACCTC AATGGTCGGT GTTACCCTG AACGCCGACC TCGACGGGTT
 27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG
 27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG
 GGCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC
 27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG
 27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG
 28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG
 GTCTCTGCCG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC
 28051 CGGGCGGCTT TCGTCACAGG GTGCGGTGCG CCGGGCAGGG TATAACTCAC
 GCCCGCCGAA AGCAGTGTC CACGCCAGCG GGCCCGTCCC ATATTGAGTG
 28101 CTGACAATCA GAGGGCGAGG TATTCAGTCT AACGACGAGT CGGTGAGCTC
 GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG
 28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GCGCCCGGCC
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG
 28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC
 CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG
 28251 TCTGAGCCGC GCTCTGGAGG CATTGGAAC TCGCAATTTA TTGAGGAGTT
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTAAAT AACTCCTCAA
 28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC
 ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG
 28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GCGGACGGC
 GCCTAGTTAA ATAAGGATTG AACTGCGCC ATTCTCTGAG CCGCCTGCCG
 28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT
 ATGCTGACTT ACAATTACCC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CCGCCGACACA AGTGCTTTGC CCGCGACTCC GGTGAGTTT
CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCCTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGC GCACGGCGTC
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTG GGGAGTTTAC
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTGCCAT
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAATATAC TGGGGCTCCT
GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG
TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG
GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT
AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT
TGAGGTAGTC TTTTGTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT
CGCAGTGGCC GCGGACGTGG TGTGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTGTGCC TCCACTCGAA

29051 AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT
TCTTTTGGGA ATCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA
CTTGTTAAGT TCGTTGAGAT GCCCATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTGGG GGTATTCTC TGTCTTGTA TTCTCTTTAT TCTTATACTA
AGCCCCAACC CCAATAAGAG ACAGAACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCTGTC TGTGTGCACA TTTGCATTTA
TGCGAAGAGA CGGATCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT
AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTAATCACC TTGCGTCAGC CCACGGTACC ACCCAAAAGG
TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT
ACCTAAAATT CTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29401 GAGTGCACCA CTTATAAA ATGCACCACA GAACATGAAA AGCTGCTTT
CTCACGTGGT GAGAATATTT TACGTGGTGT CTTGTACTTT TCGACGAATA

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTATTATGCT ATTTGGCAGC
AGCGGTGTTT TTGTTTTAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT
GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT
TTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA
CATGTACTCG TTTGTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG
TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA
CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTAATAAGTT ACAAAGCTAA TGTCAACCACT
CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA
TTGACGAAAT GAGCGACGAA CGTTTGTGTT AAGTTTTC AATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCCAGTCAT TTCCTGCTCA ATACCATTCC
TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGAATCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA
GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC
CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTGCT GGACAGGGCG

30001 GGATTGTGTC CAGTCCAACT ACAGCGACCC ACCCTAACAG AGATGACCAA
CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCGC CTACCGGACT TACATCTACC ACAAATACAC
GTGTTGGTTG CGCCGCGCGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG
GGGTTCAAAG ACGGAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG
AAGAGGTATC GCGAATACAA ACATACGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG
GACGGATTTC GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC
ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT
TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30351 TTTATATTAC T CCTTGT TGCCTTTTT TGTGCGTGCT CCAAT C
 AAATATAATG ACTGGGAACA ACGCGAAAA ACACGCACGA GGTGTAACCG
 30401 TGCAGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT
 ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTGAGATAA
 30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG
 ACGAAATGCC TAAACAGTGG GAGTGCAGT AGACGTCGGA GTAGTGACAC
 30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA
 CAGTAGCGGA AATAGGTCAC GTAACGACC CAGACACACG CGAAACGTAT
 30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA
 AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT
 30601 GAATTCTTTA ATTATGAAAT TTACTGTGAC TTTTCTGCTG ATTATTTGCA
 CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT
 30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA
 GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT
 30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG
 ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC
 30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTCTT
 GCTAGAAAGG CTTGCGACCA ATATACGTTA GTAGAGACAA TACCACAAGA
 30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTTGGCTGG
 CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAAACCGACC
 30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT
 TTGCGTTATC TACGGTACTT GGTGGGTGTA AAGGGGCGCG GCGGATACGA
 30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC
 AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG
 30951 GCCCACCTTC TCCCACCCCT ACTGAAATCA GCTACTTTAA TCTAACAGGA
 CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT
 31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC
 CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCGCG
 31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT
 TCGCGGACGA TCTTCTGCG TCCGTCGCC GGCTCGTTGT CCGGTACTTA
 31101 CAAGAGCTCC AAGACATGGT TAACTTGAC CAGTGCAAAA GGGGTATCTT
 GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA
 31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC
 AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCTTAA TGGTGGCCTG
 31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG
 TGSCGGAATC GATGTTCAAC GGTGGTTGCG CAGTCTTTAA CCACCACTAC
 31251 GTGGGAGAAA AGCCCATAC CATAACTCAG CACTCGGTAG AAACCGAAGG
 CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 A6

31301 CTGCATTAC TCTTGTG AAGGACCTGA GGATCTCTGC ACCCTTCTA
 GACGTAAGTG AGTGGAACAG TTCCTGGACT CCTAGAGACG TGGGAATAT

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA
 TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATGAT TATTTTITTT

31401 ATAATAAAGC ATCACTTACT TAAATCAST TAGCAAATTT CTGTCCAGTT
 TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC
 ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAAACTTCTT CCACAATCTA AATGGAATGT CAGTTTCCTC
 GAGGACCGAC GTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCTGTG CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC
 GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG
 CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACGTG GCCTTTTCTT ACTCCTCCCT TTGTATCCCC
 CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG
 GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC
 TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTAA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT
 GAGAGAGACC TGCTCCGGCC GTTGAATGG AGGGTTTAC ATTGGTGACA

31851 GAGCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG
 CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT
 GTGGGGAGTG TCAATGGAGT CTTGCGGATT GACACCGACG GCGCGGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCGCTAAC
 GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTC GGGGCGATTG

32001 CGTGCACGAC TCCAAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT
 GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTGACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT
 GTCTTCCTTT CGATCGGGAC GTTGTAGTC CGGGGGAGTG GTGGTGGCTA

32101 AGCAGTACCC TTACTATCAC TGCCTCACCC CCTCTAACTA CTGCCACTGG
 TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC
 ATCGAACCCG TAACTGAACT TTCTCGGGA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CTTTGCATG TAACAGACGA CCTAAACACT
 ATCCTGATTT CATGCCCGA GGAAACGTAC ATTGTCTGCT GGATTGTGA

Figure 26 AH

32251 TTGACCGTAG CTGGTCC AGGTGTGACT ATTAATAATA CTTCCTTCA
AACTGGCATC GACACAGG TCCACACTGA TAATTATTAT GAAGGAGT

32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC
TTGATTTCAA TGACCTCGGA ACCCAAACCT AAGTGTTCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTGTG TCGGGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT
GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTA TAACTCAGC CCACAACCTG GATATTAAC
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT
TGTGTTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT
CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA
TGTGTTTAGG GGAGTTTGT TTTTAACCG TACCGATCT TAACTAAGT

32701 AACAAAGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC
TTGTTCCGAT ACCAAGGATT TGATCCTGA CCGGAATCAA AACTGTCGTG

32751 AGGTGCCATT ACAGTAGGAA ACAAATAA TGATAAGCTA ACTTTGTGGA
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAACCTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT
TTTGAGTGAA ACCAGAAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTGG GCTGTTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC
AAGTCAAAC CGACAATTTC CGTCAACCG AGGTTATAGA CCTTGTCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC
TTTCACGAGT AGAATAATAT TCTAACTGC TTTACCTCA CGATGATTTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG
ACTTCCGTGT CGGATATGTT TCGGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTTATTGTA ACAGTCAGTT

33151 GTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT
CAAATGAATT TGCCTCTGTT TTGATTTGGA CATGTGATT GGTAAATGTA

Figure 26 AI

33201 AAACGGTACA C GAAACAG GAGACACAAC TCCAAGTGCA TACTCTTCT
 TTTGCCATGT GTCTTTTGTG CTCTGTGTG AGGTTACCGT ATGAGATACA
 33251 CATTTTCATG GGACTGGTCT GGCCACAAC ACATTAAATGA AATATTTGCC
 GTAAAAGTAC CCGTACCAGA CCGGTGTGTA TGTAAATTA TTTATAAACGG
 33301 ACATCCTCTT ACACTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG
 TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC
 33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT
 ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA
 33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC
 AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG
 33451 GTACCTTAAT CAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT
 CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA
 33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC
 GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG
 33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT
 TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA
 33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA
 AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTG AGGGGCCCGT
 33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT
 CGAGTGAATT CAAGTACAGC GACAGGTGCA CGACTCGGTG TCCGACGACA
 33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT
 GGTGGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGC GGATGTA
 33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA
 CCCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT
 33801 GCGCGCGAAT AAATGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC
 CGCGCGCTTA TTTGACGACG GCGCGGCGCA GGCAGGACGT CCTTATGTTG
 33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCCGCA GCATAAGGCG
 TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC
 33901 CCTTGTCTC CGGGCACAGC AGCGCACCCCT GATCTCACTT AAATCAGCAC
 GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG
 33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG
 TCATTGACGT CGTGTGCTGG TGTATAACA AGTTTTAGGG TGTACGTTT
 34001 GCGCTGTATC CAAAGCTCAT GCGGGGACC ACAGAACCCA CGTGGCCATC
 CGCGACATAG GTTTCGAGTA CCGCCCTGG TGTCTTGGGT GCACCGGTAG
 34051 ATACCACAAG CGCAGGTAGA TTAAGTGCG ACCCCTCATA AACACGCTGG
 TATGGTGTTC GCGTCCATCT AATCACCOC TGGGGAGTAT TTGTGCGACC
 34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC
 TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34151 CATATAAACC TATTAATAA CATGGCGCCA TCCACCACCA TCCTAATAA
GTATATTGG ATCTAATTT GTACCGCGGT AGGTGGTGGT AGGATTGGT

34201 GCTGGCCAAA ACCTGCCCGC CGGCTATACA CTGCAGGGAA CCGGGACTGG
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT
CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTGTTGGG

34401 ATTCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTACAT TCGGGCAGCA GCGGATGATC
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCTTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTGG TCGTAGTGTG
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCTGAAG CAAAACCAGG
TACGGTTTAC CTTGCGGCTT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TCGGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC
ACGCCCCGAC TGTGTGTCTA GACGAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGGCTCG GGTTCATGT AACTCCTTC ATGCGCCGCT GCCCTGATAA
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTGCTTC
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TCGGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG
AAAAAATAAG GTTTTCTAAT AGGTTTGGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GCGGTGSTCA AACTCTACAG CCAAAGAACA
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTCCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGACAAT GGCTTCCAAA AGGCAAACGG
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC
GGGAGTGACG GTTACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK

35101 TCTATAAACA TTAGCACC TTCAACCATG CCCAAATAAT TCTCAT G
AGATATTTGT AAGGTCGTGG AAGTTGGTAC GGCTTTATTA AGAGTAGGCG

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA
GGTGGGAAGAG TTATATAGAG ATTCTGTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA
AACATTTTGA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA
TAGTACTAAC GTTTTAAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC
TCGCCCTGTA ATTGTTTMTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC
GCGGTCCTTG GTACTGTTTT CTGGGGTGTG ACTAATACTG TGCCTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCTGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA
GCTATATTTT ACGTTCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAG GCAGGTAAGC
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTT CGTCCATTCTG

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC
AGGCCCTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTAAACATT
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA
TCTTCGGACA GAATGTTGTC CTTTTTGTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCACTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCTGTGCC GGAGTCATAA TGTAAGACTC
TCGTGGTGGC TGTGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA
CCATTTGTGT AGTCCAACATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTTGT GTATTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA
ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36051 CATACAGCGC TACAGCG GCAGCCATAA CAGTCAGCCT TACCAG LA
GTATGTCGCG AAGGTGTCGC CGTCGGTATT GTCAGTCGGA ATGGTCATT

36101 AAAGAAAACC TATTAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA
TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT

36151 GTCACAGTGT AAAAAAGGGC CAAGTGACAGA GCGAGTATAT ATAGGACTAA
CAGTGTCACA TTTTTCCTCCG GTTCACGTCT CGCTCATATA TATCCTGATT

36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG
TTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC

36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT
GCTTGGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTG AAGGAGTTA

36301 CGTCACTTCC GTTTTCCAC GTTACGTAC TTCCATTTT AAGAAAACTA
GCAGTGAAG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT

36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAACCTA CGTCACCCGC
GTTAAGGGTT GTGTATGTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG

36401 CCCGTTCCCA CGCCCCGCGC CACGTACAA ACTCCACCCC CTCATTATCA
GGGCAAGGGT GCGGGGCGCG GTGCAGTGT TGAGGTGGG GAGTAATAGT

PacI

36451 TATTGGCTTC AATCCAAAT AAGGTATATT ATTGATGATG TTAATTAAGA
ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT

36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC
TAAGCCTAGA CGTGCCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG

36551 TCGCTTCGG CCGCATCGGG ATGCCCGCGT TGCAGGCCAT GCTGTCCAGG
AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC

36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG
GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC

36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
CTTGCCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGG

36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCG
GACTGCTCGT AGTGTTTTTA GCTGCGAGT CAGTCTCCAC CGCTTTGGG

36751 ACAGGACTAT AAAGATACCA GCGTTTCCC CCTGGAAGCT CCCTCGTGCG
TGTCCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC

36801 CTCTCCTGTT CCGACCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG

36851 CTTCCGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT
GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA

36901 TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCGGT
AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

36951 TCAGCCCGAC GCGGCCT TATCCGGTAA CTATCGTCTT GAGTCG CCG
 AGTCGGGCTG GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGG TGG
 37001 CCGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
 GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCTTAA
 37051 AGCAGAGCGA GGTATGTAGG CCGTGCTACA GAGTTCTTGA AGTGGTGGCC
 TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG
 37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
 ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT
 37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA
 TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAAGTAG GCCGTTTGT
 37201 ACCACCGCTG GTAGCGGTGG TTTTCTTGTG TGCAAGCAGC AGATTACGCG
 TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCGC
 37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGCTCG
 GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC
 37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
 TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT
 37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT
 AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA
 37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT
 CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA
 37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTCG
 GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC
 37501 TGTAAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA
 ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT
 37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
 TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT
 37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG
 GGTCCGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAATAGGC
 37651 CCTCCATCCA GTCTATTAAT TGTGCGCGG AAGCTAGAGT AAGTAGTTCG
 GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC
 37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT
 GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACC
 37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
 CAGTCCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGTTGCTA
 37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTAGCTCC
 GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG
 37851 TTCGTCCTC CGATCGTTGT CAGAAAGTAA TTGGCCGAG TGTATCACT
 AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 AN

37901 CATGGTTATG CACTGC ATAATCTCT TACTGTCATG CCAATCCTAA
GTACCAATAC CCGTGTGACG TATTAAGAGA ATGACAGTAC GGTAGGCATT

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC
ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG
GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGSTC

38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT

38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTGA
ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
TACATAAATC TTTTATTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT
TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA
TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

PacI

38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)
AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 AD

MRKAd5nef MER1063
(MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

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1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGCG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGGCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGSCCC GCCTGGCATT ATGCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT

```

Figure 27A

851 CATGACCTTA T GACTTTC CTACTTGSCA GTACATCTAC GTATTGCA
GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCGT

901 TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTICA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTGAGGCG GGGTAACGTC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCCGCT
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
CCAGGTGSCA CTCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCAGGGA
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
GGACCTCTTC GTGCCCGGGT AGTGGAGGAG GTTGTGGCGG CGGTGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
GGGTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
CACTCCGGGG TCCACGGGGA CTCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGATCCA CACCCAGGGC
GGGTCTTCTC CGTCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCT
ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG
GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC
ACCTCCTCCG GTTGTCTCCG CTCTTGTGTA CGCGGCGGGT GGGGTACAGG

Figure 27B

1801 CAGCACGGCA T AGGACCC CGAGAAGGAG GTGCTGGAGT GGAGGT SA
 GTCGTGCCGT ACCTCCTGGG GCTCTTCCTC CACGACCTCA CCTCCAAGCT
 1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
 GAGGTTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA
 1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
 TGTTCTCGAC GATTTTCGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC
 1951 CCATCTGTTG TTTGCCCTC CCCCCTGCCT TCCTTGACCC TGGAAGGTGC
 GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG
 2001 CACTCCCACT GTCTTTCTCT AATAAAATGA GGAAATGCA TCGCATGTGC
 GTGAGGGTGA CAGGAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG
 2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
 ACTCATCCAC AGTAAGATAA GACCCCCCACC CCCACCCCGT CCTGTCTGTTT
 2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC
 CCCCTCTTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG
 2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
 ATACCGGCTA GCGCGCGGCG ATGACTTTAC ACACCCGCAC CGAATTCCCA
 2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGT ATCTGTTTTG
 CCCTTTCTTA TATATTCCAC CCCAGAATA CATCAAAACA TAGACAAAC
 2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATGTG
 GTCGTCCGCG GCGGCGGTAC TCGTGGTTGA GCAAACCTACC TTCGTAACAC
 2301 AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TCGTCAGAA
 TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT
 2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACCTTA
 ACACIACCCG AGGTCTGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT
 2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC
 GATGGAACTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG
 2451 TCCGCCGCG CTTCAGCCGC TGCAGCCACC GCGCGCGGA TTGTGACTGA
 AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT
 2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG
 GAAACGAAAG GACTCGGGCG AACGTTTGTG ACGTCGAAGG GCAAGTAGGC
 2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
 GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAACTGG
 2601 CGGGAACCTTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
 GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCTGTTA
 2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCA TCGGTTTAA AACATAAATA
 AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT
 2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTG TTGCTGTCTT
 TTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

Figure 27C

2751 TATTTAGGGG TTTTGGCGCG GCGGTAGGCC CGGGACCAGC GGTCTCGGTC
 ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG
 2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGA CTCTGGA
 CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT
 2851 TGTTAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
 ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG
 2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA
 ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT
 2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG
 CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC
 3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTA CAAAGCGGTT AAGCTGGGAT
 GGTCCTCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA
 3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTtaggTT
 CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA
 3101 GGCTATGTTT CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA
 CCGATACAAG GGTGCGGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT
 3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA
 GGTGCTGTCA CATAGGCCAC GTGAACCCCT TAAACAGTAC ATCGAATCTT
 3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
 CCTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG
 3251 CATGCATTCG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCTGGG
 GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCGC CGCCGGACCC
 3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA
 GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT
 3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
 AGCAGTATCC GGTAAAAATG TTTGCGGCC GCCTCCACG GTCTGACGCC
 3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA
 ATATTACCAA GGTAGGCCGG GTCCCCGAT CAATGGGAGT GTCTAAACGT
 3451 TTTCCACGCG TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG
 AAAGGGTGCG AAACCAAGT CTACCCCTC AGTACAGATG GACGCCCCCG
 3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG
 TACTTCTTTT GCCAAAGGCC CCATCCCTC TAGTCGACCC TTCTTTCGT
 3551 GTTCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC
 CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG
 3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCATCC
 GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG
 3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTT
 GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

Figure 27D

3701 CCTGACCAAA TCCAGAA GCGCTCGCC GCCCAGCGAT AGCAGTCTT
 GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA
 3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG
 CGTTCCTTCG TTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC
 3801 CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC
 GAAAACTCGC AAATGGTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG
 3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
 GACGAGATGC CGTAGAGCTA GGTCGTATAG AGGAGCAAAG CGCCCAACCC
 3901 GCGGCTTTTC CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG
 CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC
 3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTACCG
 AGTACAGAAA GGTGCCCGCG TCCAGGAGC AGTCGCATCA GACCCAGTGC
 4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT
 CACTTCCCA CGCGAGGCC GACGCGCGAC CGGTCCCACG CGAACTCCGA
 4051 GGTCCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
 CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT
 4101 GGTCGATTT GACCATGGTG TCATAGTCCA GCCCTCCGC GGCCTGGCCC
 CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCAGG
 4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
 AACCGCGCGT CGAACGGGAA CCTCTCCGC GCGGTGCTCC CCGTACGTC
 4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
 TGAAAACCTC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCCTCA
 4251 AGGCATCCGC GCCCGAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
 TCCGTAGGCG CGGCGTCCGG GCGGTCTGCC AGAGCGTAAG GTGCTCGGTC
 4301 GTGAGCTCTG GCCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT
 CACTCGAGAC CGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA
 4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA
 CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT
 4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCTG
 GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC
 4451 AGCGGTGTTC CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC
 TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG
 4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
 TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTCAAC CTCCCCATCG
 4551 GGTCGTTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
 CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCCACAC TTCTGTGTAC
 4601 TCGCCCTCTT CGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC
 AGCGGGAGAA GCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4651 GTGACCGGGT CCTGAAG GGGGGCTATA AAAGGGGGTG GGGGCCTT
CACTGGCCCA CAAGGACTTC CCCCCGATAT TTTCCCCCAC CCCCgcgcaa

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCACT
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTAC CTGGCCCCG GTGATGCCTT
AAGGTTTTTG CTCCTCCTAA ACTATAAGTG GACCGGGCG CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA
ACTCCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGACAGCA ACTTGGCGAT
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCTG TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTGCGGATC GCGCGCTCC TTGGCCGCGA
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGCGCT

5001 TGTTTAGCTG CACGTATTCT GCGCAACGC ACCGCCATTG GGGAAAGACG
ACAAATCGAC GTGCATAAGC GCGGTTGCG TGGCGGTAAG CCTTTCTG

5051 GTGGTGGCT CGTCGGGCAC CAGGTGCAG CGCAACCGC GGTGTGTCAG
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAAG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGACAAACC

5151 TCCAGCAGAG GCGGCGCCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT
AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCAGA

5201 AGCTGCGTCT CGTCCGGGG GTCTGCGTCC ACGGTAAAGA CCCCgggCAG
TCGACGCAGA GCAGGCCCC CAGACGCAGG TGCCATTTCT GGGGCCCCGTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTT AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGT GAGTGGGGGA
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC
CATTTCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA
AAGGTGGCGC CTACGACCGC GCGTGCAATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CCCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5601 GGAAGACGTT CTTGGCG TCTGTGAGAC CTACCGCGTC ACGCAO G
 CCTTCTGCAA CTTGACCGC AGACACTCTG GATGGCGCAG TCGTGCTTC
 5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC
 CTCGCGATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG
 5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT
 CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA
 5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTCGCGGTCT
 CAGGGAAAAA AAAGGTGTG AGCGCCAACCT CCTGTTTGAG AAGCGCCAGA
 5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC
 AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCCTCG
 5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA
 ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT
 5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC
 GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG
 5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT
 CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA
 6001 GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG
 CAGCAGCGTA GCGGGACGA GGGTCTCGTT TTTCAAGCAC CCGAAAAACC
 6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC
 TTGCGCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG
 6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCC GCACCTCGGA
 CGCGCTCCGT ATTTCAACGC AACTACGCC TTCCAGGGC CGTGGAGCCT
 6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA
 TGCCAAACAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT
 6201 TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG
 ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACCTAC
 6251 GAAGGCAATT TTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG
 CTTCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC
 6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA
 GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCT
 6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG
 TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC
 6401 GTCTAAACT GCGGACCTAT GGCCATTTTT TCTGGGTTGA TGCAGTAGAA
 CAGGATTGTA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT
 6451 GGTAAGCGGG TCTTGTTCCT AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT
 CCATTCGCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA
 6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACTT CATGACCAGC
 GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG

Figure 27G

6551 ATGAAGGGCA (C)CTGCTT CCCAAAGGCC CCCATCCAAG TATAGG (C)C
 TACTTCCCGT GCTCGACGAA GGGTTTCCGG GGGTAGGTTC ATATCCAGAG
 6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG
 ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC
 6651 GGAAGAAGTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG
 CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACCAG TAACTACACC
 6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA
 ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAACAT
 6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA
 TTTTGACGCG GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT
 6801 GGTTCACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTGAGCCCC
 CCAACTGGAC TGCTGGCGCG GTTCTCTCG TCTCACCCTT AACTCGGGG
 6851 TCGCCTGGCG GGTTCGGCTG GTGGTCTTCT ACTTCGGCTG CTGTCTCTTG
 ACGGACCCG CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC
 6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC
 TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGGCGCG
 6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTCCGAG CTTGATGACA
 CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT
 7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG
 TGTAGCGGCT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC
 7051 GTCAGGCGGG AGCTCCTGCA GGTTCACCTC GCATAGACGG GTCAGGGCGC
 CAGTCCGCCC TCAGGACGCT CCAAATGGAG CGTATCTGCC CAGTCCCGCG
 7101 GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGCG
 CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCAGACCA CCACCGCCGC
 7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GGC GCGACTA CGGTACCGCG
 AGCTACCGAA CGTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC
 7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCTT GGATGATGCA TCTAAAAGCG
 ,GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC
 7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA
 CACTGCGCCC GCTCGGGGCG CTCCATCCCC CCCGAGGCCT GGGCGGCCCT
 7301 GAGGGGGCAG GGGCACGTGC GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC
 CTCCCCCGTC CCCGTGCAGC CGCGGCGCGC GCCCGTCTC GACCACGACG
 7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTTGA TCTCCTGAAT
 CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA
 7401 CTGGCGCCTC TCGGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG
 GACCGCGGAG ACGCACTTCT GCTGCCCGGG CCACTCGAAC TTGGACTTTC
 7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAAA
 TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCGG GACCGCGTTT

Figure 27H

7501 ATCTCCTGCA CCTCCTGA GTTGTCTTGA TAGGCGATTG GGCEAATA
 TAGAGGACGT GAGGAGACT CAACAGAACT ATCCGCTAGA GCCGGTATT
 7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG
 GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC
 7601 TGGCGGCGAG GTCGTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
 ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC
 7651 AGGCCTCCCT CGTTCAGAC GCGGCTGTAG ACCACGCCCC CTTCCGCATC
 TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTCCGGGG GAAGCCGTAG
 7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCAGC TGCCGGGCGA
 CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT
 7751 AGACGGCGTA GTTTCGAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG
 TCTGCCGAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC
 7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
 CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG
 7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA
 CAATATAGG GGGTTCGGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT
 7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
 GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG
 7951 TCCAGAAGAC GGATGAGCTC GCGGACAGTG TCGCGCACCT CGCGCTCAAA
 AGGTCTTCTG CCTACTCGAG CCGCTGTAC AGCGCGTGGA GCGCGAGTTT
 8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT
 CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA
 8051 CCCCTTCTTC TTCTTCTGGC GGCGGTGGGG GAGGGGGGAC ACGGCGGCGA
 GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT
 8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
 GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC
 8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTCTCG CGGGGGCGCA
 CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCT
 8201 GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG
 CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCGGAC
 8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT
 GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA
 8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
 TCCATGAGGC GCGGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC
 8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG
 TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC
 8401 AGCACCCTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA
 TCGTGGCACC GCCCGCGCTC GCCCGCGGCC AGCCCCAACA AAGACCGCCT

Figure 27I

8451 GGTGCTGCTG ATGTAAT TAAAGTAGGC GGTCTTGAGA CGGCGGCG
 CCACGACGAC TACTACATTA ATTTTCATCCG CCAGAACTCT GCCGCCTTCC
 8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG
 AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC
 8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA
 AGCCGGTACG GGGTCCGAAG CAAAACGTGA GCCGCGTCCA GAAACATCAT
 8601 GTCTTGATG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTGTGTC
 CAGAACGTAC TCGGAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG
 8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG
 GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC
 8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG
 ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC
 8751 AAGCAGGGCT AGGTCCGCGA CAACCGCTC GGCTAATATG GCCTGCTGCA
 TTCGTCCCGA TCCAGCCGCT GTTGCAGGAG CCGATTATAC CGGACGACGT
 8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCACAAA GCGGTGGTAT
 GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA
 8851 GCGCCCGTGT TGATGGTGT AGTGCACTG GCCATAACGG ACCAGTTAAC
 CCGGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG
 8901 GGTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG
 CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC
 8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT
 GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA
 9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT
 GGGTGGTTTT TCACGCCGCC GCCGACGCC ATCTCCCCGG TCGCATCCCA
 9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT
 CCGGCCCGCA GGCCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA
 9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC
 TCTACATGGA CTTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG
 9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC
 CCTTTCAGCG CTTGCGCCAA GGTCTACAA GCGTCGCCGT TTTTCACGAG
 9201 CATGGTCCGG ACCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT
 GTACCAGCCC TCGAGAGCCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA
 9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT
 TCTGGCACGT TTTCTCTCG GACATTCGCC CGTGAGAAGG CACCAGACCA
 9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT
 CCTATTTAAG CGTTCACATA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA
 9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTGAAACCC
 TAGGCCGGCA GCGGCGACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9401 AGGTGTGCGA CAGACAA CGGGGAGTG CTCCTTTTGG CTTCCCTA
 TCCACACGCT GCAGTCTGTT GCCCCTCAC GAGGAAAACC GAAGGAAGGT
 9451 GGC GCGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG
 CCGCGCCGCC GACGACGCGA TCGAAAAAC CCGTGACCGG CCGCGCTCGC
 9501 TAAGCGGTGA GGCTGGAAG CGAAAGCATT AAGTGGCTCG CTCCTGTAG
 ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC
 9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC
 GGCTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGG CCAAGCTCAG
 9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA
 AGCCTGGCCG GCCTGACGCC GCTTGCCCC AAACGGAGGG GCAGTACGTT
 9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT
 CTGGGCGCAA CGTTTAAGGA GGCTTGTGTC CCTGCTCGGG GAAAAACGA
 9701 TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG
 AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGG AGGAGTCGTC
 9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCCT CCCCTCCTCC
 GCCGTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG
 9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
 ATGGCGCAGT CCTCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC
 9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG
 TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC
 9901 GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCTGAGC GGCACCCAAG
 CCGCTCCCGG ACCGCGCCGA TCCTCGCGG AGAGGACTCG CCGTGGGTTT
 9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
 CCACGTGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG
 10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG
 ACAAAGCGCT GCGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC
 10051 TTCCACGAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGTTTGCT
 AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA
 10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACC GGATT AGTCCCGCGC
 CCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG
 10151 GCGCACACGT GCGGCGCGC GACCTGGTAA CCGCATACGA GCAGACGGTG
 CCGCTGTGCA CCGCGCGCG CTGGACCATT GCGGTATGCT CGTCTGCCAC
 10201 AACCAGGAGA TTAAC TTCA AAAAGCTTT AACAACCAG TCGGTACGCT
 TTGGTCTCT AATTGAAAGT TTTTTCGAAA TTGTGTTGTC ACGCATGCGA
 10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
 ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC
 10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG
 ATTCGCGCGA CCTCGTTTTG GGTATTATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

10351 TTCCTTATAG TGCACAG CAGGGACAAC GAGGCATTCA GGGATG T
AAGGAATATC ACCTCGTGTC GTCCCTGTTG CTCCGTAAGT CCCTACGCA

10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA
CGATTTGTAT CATCTCGGGC TCCGCGCGAC CGACGAGCTA AACTATTTGT

10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG
AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCG
CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC

10551 CAAGATATAC CATACCCTT ACGTCCCAT AGACAAGGAG GTAAAGATCG
GTTCTATATG GTATGGGAA TGCAAGGTA TCTGTTCTC CATTCTAGC

10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC
TCCCCAAGAT GTACCGGTAC CCGGACTTCC ACGAATGGAA CTCGCTGCTG

10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG
GACCCGCAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC
CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTGCGAC GTTTCCCGGG

10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG
ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801 GCGGCTGACC TGGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG
CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGACC TCCGTCGACC

10851 GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG
CCGGCCTGGA CCCGACGCC ACCGTGGCG GCGCGACCG TTGCAGCCGC

10901 GCGTGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG
CGCACCTCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951 TACTAAGCGG TGATGTTCT GATCAGATGA TGCAAGACGC AACGGACCCG
ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCGCG TTGCCTGGGC

11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA
CGCCACGCCC GCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC
GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG
GACTGCCAA GCGCGTCGTC GCGTCCGGT TGGCCGAGAG GCGTTAAGAC

11151 GAAGCGGTGG TCCCGGCGCG CGAAACCCC ACGCACGAGA AGGTGCTGGC
CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TCGTGCTCT TCCACGACCG

11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG
CTAGCATTTG CCGGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC

11251 GCCTGGTCTA CGACGCGCTG CTTGAGCGCG TGGCTCGTTA CAACAGCGGC
CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTGCGCG

Figure 27L

11301 AACGTGCAGA CCTGGA CCGGCTGGTG GGGGATGTGC GCGAGG T
 TTGCACGTCT GGTGGACCT GGCCGACCAC CCCCTACACG CGCTCCGCA
 11351 GGGCGAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG
 CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC
 11401 CACTAAACGC CTTCTGAGT ACACAGCCCC CCAACGTGCC GCGGGGACAG
 GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTGCACGG CGCCCTGTG
 11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CCGCTAATGG TGACTGAGAC
 CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG
 11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA
 TGGCGTTTCA CTCCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT
 11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG
 CATCTGTTCC GGACGTCTGG CATTGGAAT CCGTCCGAAA GTTTTGAAC
 11601 CAGGGGCTGT GGGGGGTGCG GGCTCCACA GCGGACCGCG CGACCGTGTG
 GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG
 11651 TAGCTTGCTG ACGCCCAACT CGCGCTGTT GCTGCTGCTA ATAGCGCCCT
 ATCGAACGAC TCGGGTTGA GCGCGGACAA CGACGACGAT TATCGCGGGA
 11701 TCACGGACAG TGGCAGCGTG TCCCGGACA CATACTAGG TCACTTGCTG
 AGTGCTGTG ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC
 11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT
 TGTGACATGG CGCTCCGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA
 11801 CCAGGAGATT ACAAGTGTA GCCGCGCGCT GGGGAGGAG GACACGGGCA
 GGTCTCTAA TGTTCACAGT CCGCGCGCGA CCCCCTCCTC CTGTGCCCCG
 11851 GCCTGGAGGC AACCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC
 CGGACCTCCG TTGGGATTG ATGGACGACT GGTGGCCGC CGTCTTCTAG
 11901 CCCTCGTTGC ACAGTTTAA CAGCGAGGAG GAGCGCATTT TGCGCTACGT
 GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACSCGATGCA
 11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGTA ACGCCCAGCG
 CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TGCGGGTCCG
 12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA
 ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATACTGAGT
 12051 AACC GGCCGT TTATCAACCG CCTAATGGAC TACTTGCAAT GCGCGCCCGC
 TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG
 12101 CGTGAACCCC GAGTATTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC
 GCAC TTGGGG CTCATAAAGT GGTACGGTA GAACTTGGGC GTGACCGATG
 12151 CGCCCCCTGG TTCTACACC GGGGGATTG AGGTGCCCCA GGGTAACGAT
 GCGGGGGACC AAAGATGTGG CCCCCAAGC TCCACGGGCT CCCATTGCTA
 12201 GGATTCTCT GGGACGACAT AGACGACAGC GTGTTTCCC CGCAACCGCA
 CCTAAGGAGA CCCTGCTGTA TCTGCTGTCG CACAAAAGG GCGTTGGCGT

Figure 27 M

12251 GACCCTGCTA GATTGCAAC AGCGCGAGCA GGCAGAGGCG GCTCTCTTAA
 CTGGGACGAT CTAACGTG TCGCGCTCGT CCGTCTCCGC CGCGACCTT

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
 TCCTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTGAG ATGCTAGTAG CCCATTTCCT AGCTTGATAG GGTCTCTTAC
 GCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA
 GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATTT
 TGTGAGCGA CGACGTCCGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
 GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTCCGAGG CCCGCGCCCG CCCACCCGTC
 CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
 CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTTGGGA GGGAGTGGCA ACCCGTTTGC
 CGTCTGCTGT CGTCGCAGGA CCTAAACCCT CCCTCACCCT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG
 CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTCT
 TACGTTTTAT TTTTGTAGTG GTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCTCC
 ACATAAGGGG AATCATAACG CCGCGCCGCG TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
 AGGGAGGATG CTCTCACACC ACTCGCGCGC CGGTACCGC GCGCGGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTGTGCC TCCGCGGTAC
 CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCTA CCGGGGGGAG AAACAGCATC CGTACTCTG AGTTGGCACC
 GACGCCGGAT GGGCCCCCTC TTTGTCTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
 GGATAAGCTG TGGTGGGCAC ACATGGACCA CTTGTTGTTC AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
 ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
 TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
 AGAACTGCTG GCCAGCGTGA CCGCGCCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13201 CCAACATGCC AATGTGAAC GAGTTCATGT TTACCAATAA GTTTAAAGCG
 GGTGTACGG TTTCACTTG CTCAAGTACA AATGGTTATT CAAATTGTC
 13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA
 GGGCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT
 13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCCG GGGCAACTAC TCCGAGACCA
 TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT
 13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG
 ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTCAC
 13401 GGCAGACAGA ACGGGGTCTT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC
 CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACGTGT
 13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG
 GCGGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC
 13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA
 CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAGTAAAA CGACGGTCTT
 13551 TGCGGGGTGG ACTTCACCCA CAGCCGCTG AGCAACTTGT TGGGCATCCG
 ACGCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC
 13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG
 GTTCGCCGTT GGAAGGTCC TCCGAAATC CTAGTGGATG CTACTAGACC
 13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC
 TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG
 13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GCGCGAGGCG GCAGCAACAG
 AACTTTCTAC TGTGGCTTGT CCGCCCCCA CCGCGTCCGC CGTCGTTGTC
 13751 CAGTGGCAGC GGC CGGAAG AGAACTCCAA CGCGGCAGCC GCGCAATGC
 GTACCCGTCG CCGCGCCTTC TCTTGAGGT GCGCCGTCGG CGCCGTTACG
 13801 AGCCGGTGGG GACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC
 TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAACGG
 13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC
 TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTGCGC GGCTTCGACG
 13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA
 GCGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT
 13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC
 AGTTTGGGGA CTGTCTCCTG TCGTCTTTTG CGTCAATGTT GGATTATTG
 14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACACTA
 TTACTGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT
 14051 CGGCGACCCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACTCCTG
 GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC
 14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTGCTTGGC AGACATGATG
 TGCAATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14151 CAAGACCCCG TTTTCCG CTCCACGCGC CAGATCAGCA ACTTTC
 GTTCTGGGGC ACTGGAAGGC GAGGTGCGCG GTCTAGTCGT TGAAAGGCA
 14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC
 CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG
 14251 AGGCCGTCTA CTCCCACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG
 TCCGCGAGAT GAGGCTTGAG TAGGCGCTCA AATGGAGAGA CTGGGTGCAC
 14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAC
 AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CCGCGGGGCG GTGGGGGTG
 14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC
 GTAGTGGTGG CAGTCACCTT TGCAAGGACG AGAGTGCTTA GTGCCCTGCG
 14401 TACCGCTGCC CAACAGCATC CGAGGAGTCC AGCGAGTGAC CATTACTGAC
 ATGGCGACGC GTTGTCTGAG CCTCCTCAGG TCGCTCACTG GTAATGACTG
 14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC
 CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG
 14501 GCGCGCGGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA
 CGCGCGCAG GATAGCTCGG CGTGAAAAC TCGTTCGTAC AGGTAGGAAT
 14551 TATCGCCAG CAATAACACA GGCTGGGGCC TCGGCTTCCC AAGCAAGATG
 ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC
 14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGC GCGG
 AAACCGCCCC GGTCTTTCGC GAGGCTGGTT GTGGGTCACG CGCACGCGCC
 14651 GCACTACCGC GCGCCCTGGG GCGCGCACA ACGCGGCCGC ACTGGGCGCA
 CGTGATGGCG CGCGGGACCC CGCGCTGTT TCGCGCGGCG TGACCCGCGT
 14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC
 GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG
 14751 ACGCCACGCG CGCCACCACT GTCCACAGTG GACGCGGCCA TTCAGACCGT
 TCGGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA
 14801 GGTGCGCGGA GCCCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG
 CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC
 14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG
 ATCGTGACAGC GGTGGCGGCG GCTGGGCCGT GACGCGGGGT TCGCGCGCGC
 14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GCGCGACGGG CGGCCATGCG
 CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC
 14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA
 CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT
 15001 GGCGACGAGC GGCCGCGGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG
 CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAAATCAG ATACTGAGTC
 15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCTGCG
 CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGACGC

Figure 27P

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15101 CGTGCCCGTG CCGCCCGCC CCCCAGCGCAA CTAGATTGCA AGAAAAAT
GCACGGGCAC GCGGGGCGG GGGGCGCGTT GATCTAACGT TCTTTTAA

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA
TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA ACAGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC
CGATACAGGT TCGCGTTTTA GTTCTCTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA
CCTCTAGATA CCGGGGGGCT TCTTCTTCTC CGTCTAATG TTCGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAACCTGAC
TCGATTTGCG CCAGTTTTC TTTTCTTTC TACTACTACT ACTTGAAC TG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCA C

15401 GAAAGGTGCA CGCGTAAAAC GTGTTTTCG ACCCGGCACC ACCGTAGTCT
CTTTCCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCCG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG
AATGCGGGCC ACTCGCGAGG TGGCGTGGG TGTTCGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCC T

15551 GTTTCCTTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG
CAACCGGATG CTTTTCGCCG TATTCTGTG CGACCGCAAC GCGGACCTGC

15601 AGGGCAACCC AACACCTAGC CTAAGCCCCG TAACACTGCA GCAGGTGCTG
TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCTTAAAGC GCGAGTCTGG
GGGCGCGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTG CGCTCAGACC

15701 TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG
ACTGAACCGT GGGTGGCAGC TCGACTACCA TGGGTTGCGG GTCGCTGACC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC
TTCTACAGAA CCTTTTTCAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGA CTGGGCG TGCAGACCGT
GCGCACGCGG GTTAGTTCGT CCACGCGGCG CCGACCCGC ACGTCTGGCA

15851 GGACGTTTCA ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG
CCTGCAAGTC TATGGGTGAT GGTCACTCGT GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGCTTG CCTCAGCGGT GCGGATGCC
TCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCTACGG

15951 GCGGTGCAGG CGGTCGCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA
CGCCACGTCC GCCAGCGAGC CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGCGC CCGCGCGGTT
TTGCTTGGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GCGCGGCAA

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Figure 27A

16051 CGAGGAAGTA CCGGCGGCC AGCGCGCTAC TGCCCGAATA TGCCCTAAT
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT
CAGCGGCAGC GGTGCGGCAC GACCGGGGCT AAAGGCACGC GTCCACCCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG
GCGCTTCCTC CGTCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA
TCCCCGTACC GGCCGGTGCC GGACTGCCCG CCGTACGCAG CACCGGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTGCGAT GCGCGGCGGT ATCCTGCCCC
GGCCGCGGCC GCGCGCAGCG TGGCAGCGTA GCGCGCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA
AGGAATAAGG TGA CTAGCGG GCGCGCTAAC GCGGCGACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TAAAAACAA GTTGCAATGTG
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGTG CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGA CTCTC ACGCTCGCTT GGTCTGTAA
CTTTTGTAGT TTATTTTCTA GACCTGAGAG TCGGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCGCGCGACA
GATAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT
ACTCGCCACC GCGGAAGTCG ACCCGAGCG ACACCTCGCC GTAATTTTTA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGG CCTGGCCAAC
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTGATCCCC GCCCTCCCGT
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17001 AGAGGAGCCT CCGGCCG TGGAGACAGT GTCTCCAGAG GGGCGTGG
 TCTCCTCGGA GGTGGCCGGC ACCTCTGTCA CAGAGGTCTC CCCGCACCGC
 17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC
 TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG
 17101 GAGCCTCCCT CGTACGAGGA GGCATAAAG CAAGGCCTGC CCACCACCCG
 CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGGC
 17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA
 AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCTGT TGTGGGCATT
 17201 CGCTGGACCT GCCTCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA
 GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT
 17251 GGGCCGACCG CCGTGTGTGT AACCCGTCTT AGCCGCGCGT CCCTGCGCCG
 CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACCGCGC
 17301 CGCCGCCAGC GGTCCGCGAT CGTTCCGGCC CGTAGCCAGT GGCACCTGGC
 GCGCGGTCTG CCAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG
 17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC
 TTTCTGTGA CTGTCTGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG
 17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC
 GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG
 17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG
 GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGGGG CGAAAGGTTT
 17501 ATGGCTACCC CTTCGATGAT GCCGCAGTGG TCTTACATGC ACATCTCGGG
 TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC
 17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGACG TTTGCCCGCG
 GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC
 17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG
 GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC
 17651 CGCCTACGC ACGACGTGAC CACAGACCGG TCCAGCGTT TGACGCTGCG
 CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTCCGACCG
 17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT
 CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA
 17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC
 AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG
 17801 TTTGACATCC GCGGCGTGCT GGACAAGGGC CCTACTTTTA AGCCCTACTC
 AAAGTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG
 17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGTGCCCCA AATCCTTGCG
 ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC
 17901 AATGGGATGA AGCTGCTACT GCTCTTGAAA TAAACCTAGA AGAAGAGGAC
 TTACCCTACT TCGACGATGA CGAGAACTT ATTTGGATCT TCTTCTCCTG

Figure 275

17951 GATGACAACG ACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAA
 CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTGTGAGT
 18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA
 GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT
 18051 TTCAAATAGG TGTCGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT
 AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAA
 18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA
 GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT
 18151 TCATGCGAGT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT
 AGTACGTCGA CCCTCTCAGG ATTTTCTCTG ATGGGGTTAC TTTGGTACAA
 18201 ACGGTTTCTA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT
 TGCCAAGTAT ACGTTTGGG TGTCTTACTT TACCTCCCGT TCCGTAAGAA
 18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAG TGCAATTTTT
 CATTTCTGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA
 18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG
 GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC
 18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT
 ACCATAACAT GTCACCTTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA
 18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA
 AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT
 18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA
 TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAAA
 18501 TTGGTCTAAT GTATTACAAC AGCAGGGGTA ATATGGGTGT TCTGGCGGGC
 AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG
 18551 CAAGCATCGC AGTTGAATGC TGTGTAGAT TTGCAAGACA GAAACACAGA
 GTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT
 18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT
 CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA
 18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT
 AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA
 18701 ATTGAAAATC ATGGAAGTGA AGATGAACTT CCAAATTACT GCTTTCCACT
 TAACTTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA
 18751 GCGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAACAG
 CCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC
 18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAA
 CAGTCCTTTT ACCTACCCTT TTTCTACGAT GTCTTAAAAG TCTATTTTAA
 18851 GAAATAAGAG TTGGAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA
 CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18901 CCTGTGGAGA A TCCTGT ACTCCAACAT AGCGCTGTAT TTGCCC A
 GGACACCTCT TTAAGGACA TGAGGTTGTA TCGCGACATA AACGGGCTGT
 18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC
 TCGATTTTCAT GTCAGGAAGG TTGCATTTT AAAGACTATT GGGTTTGTGG
 19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA
 ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT
 19051 CATTAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC
 GTAATTGGAA CCTCGTGCGA CCAGGGAAC TATATACCTG TTGCAGTTGG
 19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG
 GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC
 19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT
 CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA
 19201 TGCCATTAAG AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA
 ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT
 19251 ACTTCAGGAA GGATGTTAAC ATGGTCTGTC AGAGCTCCCT AGGAAATGAC
 TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG
 19301 CTAAGGGTTG ACGGAGCCAG CATTAGTTT GATAGCATTT GCCTTTACGC
 GATTCCCAAC TGCTCGGTC GTAATTCAA CTATCGTAA CGGAAATGCG
 19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC
 GTGGAAGAAG GGGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG
 19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC
 AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG
 19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT
 TTGTACGAGA TGGGATATGG GCGGTTGCGA TGTTGTCACG GGTATAGGTA
 19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCTTTC ACGCGCCTTA
 GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT
 19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC
 TCTGATTCCT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG
 19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC
 ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG
 19651 CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA
 GAAATTCCTT CACCGGTAAT GGAACTGAG AAGACAGTCG ACCGGACCGT
 19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC
 TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTCGC GAGTCAACTG
 19751 GGGGAGGGTT ACAACGTTGC CCAAGTGAAC ATGACCAAAG ACTGGTTCCCT
 CCCCTCCCAA TGTGCAACG GGTACATTG TACTGGTTTC TGACCAAGGA
 19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGCG TTCTATATCC
 CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA C GACCGC ATGTACTCCT TCTTTAGAAA CTTCCA
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGA TGATACTAAA TACAAGGACT ACCAACAGGT
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTGGC TACCTTGCCC
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT
GGTGGTACGC GCTTCTGTG CCGATGGGAC GATTGAAGGG GATAGCGGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA
TATCCGTTCT GCGCTCAACT GTCGTAATGG GTCTTTTCA AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG
AGCGTGGGAA ACCGCTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCCG

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAATC CGCCACGCG
GTGAGTGTCT GGACCCGTT TTGGAAGAGA TCGGTTGAG GCGGGTGC

20201 CTAGACATGA CTTTGTAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA
GATCTGTACT GAAACTCCA CCTAGGGTAC CTGCTCGGGT GGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG
ACAAAACAAA CTTAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGGCG

20301 GCGTCATCGA AACCCTGTAC CTGCGCACGC CTTCTCGGC CGGCAACGCC
CGCAGTAGCT TTGGCATATG GACGCGTGCG GGAAGAGCCG GCCGTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC
TGTGTATTT CTTCTGTTCT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGTT GTGGGCCATA
TCACTCGTCC TTGACTTTG GTAACAGTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGCGCG GTCGCGAGAC TGGGGCGTA
TCGAGCCGAC GCGGTATCAG TTATGCCGCG CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTGTG CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTG
ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCAGCCG
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTCT CCACGCCTTT GCCAACTGGC
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20801 CCCAAACTCC C GATCAC AACCCACCA TGAACCTTAT TACCGG A
 GGGTTTGAGG GTACCTAGTG TTGGGGTGGT ACTTGGAATA ATGGCCCAT
 20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCACCC TGCGTCGCAA
 GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT
 20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA
 GGTCTTGTGTC GAGATGTGCA AGGACCTCGC GGTGAGCGGG ATGAAGCGCT
 20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTCA CTTGAAAAAC
 CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG
 21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA
 TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCTT TTACGAAAAAT
 21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG
 AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC
 21101 TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG
 AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC
 21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAACT CAGGCACAAC
 CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTGTA GTCCGTGTTG
 21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA
 GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT
 21251 CCAACGCGTT TAGCAGGTGCG GCGCCGATA TCTTGAAGTC GCAGTTGGGG
 GGTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAAGTTCAG CGTCAACCCC
 21301 CCTCCGCCCT GCGCGCGCGA GTTGCATAC ACAGGGTTGC AGCACTGGAA
 GGAGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT
 21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCAGCTC TTGTGCGAGA
 GTGATAGTCG CGGCCACCA CGTGCAGCCG GTCGTGCGAG AACAGCCTCT
 21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC
 AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG
 21451 TTTGGTAGCT GCCTTCCAA AAAGGGCGCG TGCCAGGCT TTGAGTTGCA
 AAACCATCGA CGGAAGGGT TTTCCGCGC ACGGGTCCGA AACTCAACGT
 21501 CTCGCACCST AGTGGCATCA AAAGGTGACC GTGCCCGGTC TGGGCGTTAG
 GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC
 21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC
 CTATGTCGCG GACGTATTTT CGGAAC TAGA CGAATTTTCG GTGGACTCGG
 21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT
 AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA
 21651 GGCCGACAG GCCGCGTCGT GCACGCAGCA CTTGCGTCG GTGTGGAGA
 CCGGCCTGTC CGGCGCAGCA CGTGCCTGCT GGAACGCAGC CACAACCTCT
 21701 TCTGCACCAC ATTTTCGGCC CACCGGTTCT TCACGATCTT GGCCTTGCTA
 AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27W

21751 GACTGCTCCT TCGCGCG CTGCCCCGTTT TCGCTCGTCA CATCCATC
CTGACGAGGA AGTCGCGCGC GACGGGCAAA AGCGAGCAGT GTAGGTAAAG

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT
TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCTGA

21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC
CGGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTCGCGCT CGGGCACCCG

21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG
AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TGCGGACGTC

21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT
CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA

22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCATAC GGCCGCCAGA
CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGCGCGTCT

22051 GCTTCCACTT GGTGAGGCG TAGTTTGAAG TTCGCCTTTA GATCGTTATC
CGAAGGTGAA CCACTCCGTC ATCAAACTTC AAGCGGAAAT CTAGCAATAG

22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC
GTGACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGAAGAGGG

22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT
TGCCTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA

22201 TCCGCTTCGC TGGGCTCTTC CTCTTCTCT TCGCTCCGCA TACCACGCGC
AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTTCGCG

22251 CACTGGGTCTG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC
GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG

22301 CATGCTTGAT TAGCACCGGT GGTTTGCTGA AACCACCAT TTGTAGCGCC
GTACGAACTA ATCGTGGCCA CCAACGACT TTGGGTGGTA AACATCGCGG

22351 ACATCTTCTC TTTCTTCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG
TGTAAGAAG AGAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG
CGCGAGCCCG AACCTCTTC CCGCGAAGAA AAAGAAGAAC CCGCGTTACC

22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC
GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG

22501 AGCGCGTCTT GTGATGAGTC TTCTCGTCC TCGGACTCGA TACGCCGCCT
TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA

22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG
GTAGGCGAAA AAACCCCGC GGGCCCTCC GCGCGCGTG CCCCTGCCCC

22601 ACGACACGTC CTCCATGGTT GGGGACGTC GCGCCGACC GCGTCCGCGC
TGCTGTGCAG GAGGTACCAA CCCCTGCAG CGCGGCGTGG CGCAGGCGCG

22651 TCGGGGGTGG TTTGCGGCTG CTCTCTTCC CGACTGGCCA TTTCTTCTC
AGCCCCACC AAAGCGCGAC GAGGAGAAG GCTGACCGGT AAAGGAAGAG

Figure 27X

22701 CTATAGGCAG AAGATCA TGGAGTCAGT CGAGAAGAAG GACAGC
 GATATCCGTC TTTTCTAGT ACCTCAGTCA GCTCTTCTTC CTGTCCGATT
 22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG
 GCGGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC
 22801 CCTACCACCT TCCCCGTCGA GGCACCCCGG CTTGAGGAGG AGGAAGTGAT
 GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA
 22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG
 ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC
 22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAACGAG
 ATGTTGTCTT CCTATTTTTC GTTCTGGTCC TGTGCGTCT CCGTTTGCTC
 22951 GAACAAGTCG GCGGGGGGGA CGAAGGCAT GCGGACTACC TAGATGTGGG
 CTTGTTTCAGC CCGCCCCCTT GCTTTCCTTA CCGCTGATGG ATCTACACCC
 23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG
 TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTACGCGG TAATAGACGC
 23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC
 TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG
 23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA
 GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT
 23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT
 TCTTTTGCCG TGTACGCTCG GGTGGGCGC GGAGTTGAAG ATGGGGCATA
 23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAACTGC
 AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTTTTGACG
 23251 AAGATACCCC TATCTGCGG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT
 TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTCGTCGA
 23301 GGCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG
 CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC
 23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC
 ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTG
 23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT
 CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA
 23451 GGAACGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG
 CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC
 23501 AGGTCACCCA CTTTGCTTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG
 TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC
 23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG
 TCGTGTCACT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC
 23601 GGATGCAAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG
 CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

23651 ACGAGCAGCT ACGCTGG CTTCAAACGC GCGAGCCTGC CGACTT G
TGCTCGTCGA TCGCGCGACC GAAGTTTGGC CGCTCGGACG GCTGAACCTC

23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG
TTTGTAAAGT GATGTGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTT

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG
GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGTACACC
GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT
ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG
GTTCTCTGAC GTCTTTGACG ATTTGTTTTT GAACTTCCTG GATACCTGCC

24101 CTTTCAACGA GCGCTCCGTG GCCGCGCACG TGGCGGACAT CATTTTCCCC
GGAAGTTGCT CCGGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAACCCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA
CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT
TTCTTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAAGTAC
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG
GTTGATGGAA CGGATGGTGA GACTGTATTA CTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTCGCAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTGA
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT
TTGAGTGAGG CCCCGACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 27 Z

24601 GAGGACTACC AC~~CC~~CCACGA GATTAGGTTT TACGAAGACC AATCCCC~~CC~~
 CTCTGTATGG TCGGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG
 24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG
 CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC
 24701 GCCAATTGCA AGCCATCAAC AAAGCCCCGC AAGAGTTTCT GCTACGAAAG
 CGGTTAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC
 24751 GGACGGGGGG TTTACTTGGA CCCCAGTCC GCGGAGGAGC TCAACCCAAT
 CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA
 24801 CCCCCCGCCG CCGCAGCCCT ATCAGCAGCA GCCCGGGGCC CTGCTTCCC
 GGGGGCGCGC GCGCTCGGGA TAGTCGTCGT CGGCGCCCGG GAACGAAGGG
 24851 AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA
 TCCTACCGTG GGT~~TTTT~~CTT CGACGTCGAC GGCGGCGGTG GTGCTCTGCT
 24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGT~~TTTT~~TGGAC GAGGAGGAGG
 CCTCCTTATG ACCCTGTGAG TCCGTCTCCT CCAAAACCTG CTCCTCCTCC
 24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC
 TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG
 25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTGCGAT TCCCCTCGCC
 CTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG
 25051 GGCGCCCCAG AAATCGGCAA CCGGTTCAG CATGGCTACA ACCTCCGCTC
 CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG
 25101 CTCAGGCGCC GCCGGCACTG CCCGTTCCGC GACCCAACCG TAGATGGGAC
 GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTGGC ATCTACCTGC
 25151 ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA
 TGGTGACCTT GGTCCCAGCC ATTCAGGTTT GTCGGCGGCG GCAATCGGGT
 25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG
 TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTTCTTGC
 25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTCGCCCGC
 GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG
 25301 CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCCGTA ACATCCTGCA
 GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT
 25351 TTTACTACCGT CATCTCTACA GCCATACTG CACCGCGGGC AGCGGCAGCA
 AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCGG TCGCCGTCGT
 25401 ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC
 TGTGCTCGCC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG
 25451 AAAGCCCAAG AAATCCACAG CGCGGGCAGC AGCAGGAGGA GGAGCGCTGC
 TTTCGGGTTT TTTAGGTGTC GCCGCCGTCG TCGTCTCCTT CCTCGCGACG
 25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT
 CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCTTAA

Figure 27. AA

25551 TTTCCCACTC TTTGCTAT ATTTCAACAG AGCAGGGGCC AAGAACA
 AAAGGGTGAG ACATACGATA TAAAGTGTGTC TCGTCCCCGG TTCTGTCTCT
 25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT
 CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA
 25651 ATCACA AAAAG CGAAGATCAG CTTGCGCGCA CGCTGGAAGA CGCGGAGGCT
 TAGTGT TTTT GCTTCTAGTC GAAGCGCGCT GCGACCTTCT GCGCCTCCGA
 25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT
 GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCTTGATCA AAGCGCGGGA
 25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG
 AAGAGTTTAA ATTGCGGCTT TTGATGCAGT AGAGGTGCGC GGTGTGGGCC
 25801 CGCCAGCACC TGTGTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC
 GCGGTGCTGG ACAACAGTCG CGGTAATACT CGTTCTTTTA AGGGTGCGGG
 25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCC
 ATGTACACCT CAATGGTGGG TGTTTACCTT GAACGCGGAC CTCGACGGGT
 25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT
 TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA
 25951 CCCGGGTCAA CGGAATACGC GCCCACCGAA ACCGAATTCT CCTGGAACAG
 GGGCCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC
 26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC
 CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG
 26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC
 GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG
 26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAATCAGG GCGCGAGCTT
 GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA
 26151 GCGGGCGGCT TTCGTACAG GGTGCGGTGCG CCCGGGCAGG GTATAACTCA
 CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT
 26201 CCTGACAATC AGAGGGCGAG GTATTAGCT CAACGACGAG TCGGTGAGCT
 GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA
 26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC
 GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCGG
 26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAATCTGCG AGACCTCGTC
 GCGAGAAGTA AGTCCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG
 26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT
 GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA
 26401 TTGTGCCATC GGTCTACTTT AACCCCTTCT CGGGACCTCC CGGCCACTAT
 AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA
 26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG
 GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTTCTTGA GCCGCTGCC

Figure 27 AB

26501 CTACGACTGA A TAAGTG GAGAGGCAGA GCAACTGCCG CTGAAA C
GATGCTGACT TACAATTCAC CTCCTCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCCG GGATCATATC GAGGGCCCCG CGCACGGCGT
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCTGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA
GGCCGAATGG CGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTCTCACT
GGTCTCGCGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCTTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAATATA CTGGGGCTCC
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG
ATAGCGGTAG GACATTTGCG GTGCAGAAAG TGGCGGGTT CGTTTGTTT

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA
CGCTTGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCGTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT
ACGCAGTGGC CGGCACGCTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT
AAAAGGCCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCTG CTGTGTGCAC ATTTGCATTT
TTGCCAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCTAGT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA
TAACAGTCGA AAAATTGCG ACCCCAGCGG TGGGTCTAC TAATCCATGT

27401 TAATCTTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTT

Figure 27AC

27451 GTGGATTTTA A G C C A G C CTGTAATGTT ACATTGCGAG CTGAAG A
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTGGCAG
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTCCAGG GTAAAAGTCA
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAA TTGTGTGGAA
ACATGTACTC GTTGTCTATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAATAACAA AAGCAGACGC AGCTTTATTG
CCAGACATGG GATGAGATAT AATTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT
ATTGACGAAA TGAGCGACGA ACGTTTGTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATT
TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA
GGGACTTGTT AACTGAGATA CACCTATAC GAGGTGCGA TGTGGAAC

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCACTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA
GCCTAAACAA GGTGAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCCTTGT CAATAACTGG GATAACTTGG GCATGTGGTG
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCACCAC CCATCTATAG TCCCATCATT
CGACGGATTT CGCGTTTGGC CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAAATCCAT AGATTGGACG GACTGAAACA
CACGATGTGG GTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28401 CATGTTCTTT TTTTACAG TATGATTAAA TGAGACATGA TTCCTC
 GTACAAGAAA AGAGAATGTC ATACTAATTT ACTCTGTACT AAGGAGCTCA
 28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG
 AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC
 28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT
 GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA
 28551 TTGCTTTACG GATTTGTAC CCTCACGCTC ATCTGCAGCC TCATCACTGT
 AACGAAATGC CTAAACAGTG GGAGTGCGAG TAGACGTCGG AGTAGTGACA
 28601 GGTCAATGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT
 CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA
 28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT
 TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA
 28701 AGAATTCCTT AATTATGAAA TTTACTGTGA CTTTCTGCT GATTATTGCG
 TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG
 28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC
 TGGGATAGAC GCAAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG
 28801 ATGCAGATTC ACTCGTATAT GGAATATPCC AAGTTGCTAC AATGAAAAAA
 TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT
 28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC
 CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG
 28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG
 ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAAACCGAC
 28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC
 CTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG
 29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCAGC CAATCAGCCT
 AAGGTGACGT TGTTCACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA
 29051 CGCCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG
 GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAT TAGATTGTCC
 29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG
 TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC
 29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA
 GTGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT
 29201 TCAAGAGCTC CAAGACATGG TTAAGTTGCA CCAGTGCAAA AGGGGTATCT
 AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTACGTTT TCCCCATAGA
 29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA
 AAACAGAGCA TTTCGTCCGG TTTCAGTGA TGCTGTCATT ATGGTGGCCT
 29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT
 GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27 A E

29351 GGTGGGAGAA A CATT A CCATAACTCA GCACTCGGTA GAAACC G
CCACCTCTT TTCGGTAAT GGTATTGAGT CGTGAGCCAT CTTTGGCTTC

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT
CGACGTAAGT GAGTGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAA
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT
TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT
GGAGGACCGA CGTTTGAAAG AGGTGTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC
GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACG TGCTTTTCT TACTCCTCCC TTTGTATCCC
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGT TCAAGAGAGT CCCCCTGGGG TACTCTCTTT GCGCCTATCC
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG
CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAT GTAACCACTG
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTGGA CTTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGACCC
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGACGA CTCCAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG
GGCAGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTCAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGGCCCTCA CCACCACCGA
AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA
CATCGAACCC GTAACGTAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30301 CTAGGACTAA ACGGGGC TCCTTTGCAT GTAACAGACG ACCTAA C
 GATCCTGATT TCGCCCCG AGGAAACGTA CATGTGCTGC TGGATTGTC
 30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC
 AACTTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG
 30401 AAATAAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA
 TTTGATTTCA ATGACCTCGG AACCCAAAAC TAAGTGTTCG GTTATACGTT
 30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT
 GAATTACATC GTCCTCCTGA TTCCTAATA AGAGTTTTGT CTGCGGAATA
 30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC
 TGAATAACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG
 30551 TAGGACAGGG CCCTCTTTTT ATAAACTCAG CCCACAACCT GGATATTAAAC
 ATCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG
 30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CAAAAAGCT
 ATGTTGTTTC CGGAAATGAA CAAATGTGCA AGTTTGTTAA GGTTTTTCGA
 30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA
 ACTCCAATTG GATTCGTGAC GGTCCCCAA CTACAACTG CGATGTCGGT
 30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA
 ATCGGTAATT ACGTCCTCTA CCCGAACCTA AACCAAGTGG ATTACGTGGT
 30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTC
 TTGTGTTTAG GGGAGTTTTC TTTTAAACCG GTACCGGATC TTAAACTAAG
 30801 AAACAAGGCT ATGTTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA
 TTTGTTCCGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAATGTGCTG
 30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG
 GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTGCA TTGAAACACC
 30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC
 TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG
 30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG
 ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC
 31001 TTTCACTTTT GGCTGTTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT
 AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCOA
 31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA
 GTTTCACGAG TAGAATAATA TTCTAACTG CTTTACCTC ACGATGATTT
 31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA
 GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT
 31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA
 GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT
 31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA
 CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AAGGAGACA AAACATAACC TGTAACACTA ACCATTAC
 TCAAAATGAAT TTGCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG
 31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG
 ATTTGCCATG TGTCTTTGT CCTCTGTGT GAGGTCACG TATGAGATAC
 31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC
 AGTAAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG
 31401 CACATCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT
 GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAAA
 31451 GTGTTATGTT TCAACGTGTT TATTTTTCAT TTGCAGAAAA TTTCAAGTCA
 CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT
 31501 TTTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC
 AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG
 31551 CGTACCTTAA TCAAACTCAC AGAACCTTAG TATTCAACCT GCCACCTCCC
 GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG
 31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCAGGCTGG CCTTAAAAAG
 AGGGTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC
 31651 CATCATATCA TGGGTAAACG ACATATTCTT AGGTGTATA TTCCACACGG
 GTAGTATAGT ACCCATGTG TGTATAAGAA TCCACAATAT AAGGTGTGCC
 31701 TTTCCTGTG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC
 AAAGGACAGC TCGGTTTGGC AGTAGTCACT ATAATTATTT GAGGGGCCCG
 31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG
 TCGAGTGAAT TCAAGTACAG CGACAGGTG ACGACTCGGT GTCCGACGAC
 31801 TCCAACCTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA
 AGGTGGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT
 31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC
 ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTG
 31901 AGCGCGCGAA TAAACTGCTG CCGCGCGCGC TCCGTCTGTC AGGAATACAA
 TCGCGCGCTT ATTTGACGAC GCGCGCGCGC AGGCAGGACG TCCTTATGTT
 31951 CATGGCAGTG GTCTCCTCAG CGATGATTG CACCGCCCGC AGCATAAGGC
 GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG
 32001 GCCTTGTCTT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA
 CGGAACAGGA GGCCCGTGTG GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT
 32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA
 GTCATTGACG TCGTGTGCTG GTGTTATAAC AAGTTTTAGG GTGTACGTT
 32101 GGCCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT
 CCGCGACATA GGTTCGAGT ACCGCCCTG GTGTCTTGGG TGCACCGGTA
 32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG
 GTATGGTGTG CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32201 GACATAAACA TCTCTTT TGGCATGTTG TAATTCACCA CCTCCC A
 CTGTATTGT AATGGAGAAA ACCGTACAAC ATTAAGTGGT GGAGGGCCAT
 32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC
 GGTATATTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTG
 32301 AGCTGGCCAA AACCTGCCCC CCGGCTATAC ACTGCAGGGA ACCGGGACTG
 TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC
 32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT
 CTTGTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA
 32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC
 GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG
 32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGGAACAACC
 AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTGG
 32501 CATTCCTGAA TCAGCGTAA TCCCACTG CAGGGAAGAC CTCGCACGTA
 GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT
 32551 ACTCACGTTG TGCATTGTCA AAGTGTACA TTCGGGCAGC AGCGGATGAT
 TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA
 32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC
 GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTCCTCC ATCTGCTAGG
 32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTG GTCGTAGTGT
 GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAAC CAGCATCACA
 32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTTGAA GCAAAACCAG
 GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTGGTC
 32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG
 CACGCCCGCA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC
 32801 CTCTGTGTAG TAGTTGTAGT ATATCCAATC TCTCAAAGCA TCCAGGCGCC
 GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCCGG
 32851 CCCTGGCTTC GGGTTCATG TAACTCCTT CATGCGCCGC TGGCCTGATA
 GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGCGC ACGGCACTAT
 32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCGTT
 TGTAGGTGGT GCGTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA
 32951 CTGCGAGTCA CACACGGGAG GAGCGGAAG AGCTGGAAGA ACCATGTTTT
 GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA
 33001 TTTTTTTATT CAAAAGATT ATCCAAAACC TCAAATGAA GATCTATTAA
 AAAAAATAA GGTTTTCTAA TAGGTTTGG AGTTTACTT CTAGATAATT
 33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAATCTACA GCCAAAGAAC
 CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG
 33101 AGATAATGGC ATTTGTAAGA TGTGACAAA TGGCTTCAA AAGGCAAACG
 TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33151 GCCCTCACGT GTGGAC GTAAAGGCTA AACCCCTCAG TGTGAATTC
CGGGAGTGCA GTTCACCTG CATTTCGGAT TTGGGAAGTC CCACCTTAG

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC
GAGATATTTG TAAGGTCGTG GAAGTTGGTA CCGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC
CGGTGAAGA GTTATATAGA GATTCTTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGAACTCGG AGTTCGTCTG

33351 AATCATGATT GCAAAAATTC AGGTTCTCTCA CAGACCTGTA TAAGATTCAA
TAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG
TTCGCCCTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC
GGTCGACTTG TATTAGCAGC TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCACCA CTGATTATGA CACGCATACT
GGCGGTCTTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGCG
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTGGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC
CGCTATATTT TACGTTCAC GACGAGTTT TAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG
TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG
GAGGCCCTTG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CCGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT
GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCTTATAAG CATAAGACGG
ATCTTCGGAC AGAATGTTGT CTTTTTGTG GGGAATATTC GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA
TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCACTG GCACTAATTT

33901 AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT
TTCTGCGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG
GCCATTTGTG TAGTCCAAC TAAAGTAGCC AGTCACGATT TTTCGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC
GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTGT TGTATTGTG

Figure 27A J

34101 CTGAAAAACC CCGTTGCCTA GGCAAAATAG CACCCTCCCG GTCACATAA
 GACTTTTITGG GACGAT CCGTTTATC GTGGGAGGGC GAGGCTCTT

34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA
 TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT

34201 AAAAGAAAAC CTATTAAAA AACACCACTC GACACGGCAC CAGCTCAATC
 TTTCTTTTG GATAATTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA
 TCAGTGTAC ATTTTTTCCC GGTTCACGTC TCGTCTATAT ATATCCTGAT

34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
 TTTTACGCG ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTGGCGTG

34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCAAA
 CGCTTGGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401 TCGTCACTTC CGTTTTCCTA CGTTACGTCA CTTCCCATTT TAAGAAAAT
 AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTGA

34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
 TGTAAAGGGT TGTGTATGTT CAATGAGCGG GGATTTTGGA TGCAGTGGGC

34501 CCCCCTTCCC ACGCCCCGCG CCACGTCAAA AACTCCACCC CCTCATTATC
 GGGGCAAGGG TCGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

PacI

34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
 TATAACCGAA GTTAGGTTT ATTCCATATA ATAATACTA CAATTAATTC

34601 AATTCGGATC TCGACGCGA GGCTGGATGG CCTTCCCAT TATGATTCTT
 TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651 CTCGCTTCCG GCGGCATCGG GATGCCCCG TGCAGGCCA TGCTGTCCAG
 GAGCGAAGGC CGCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
 CGTCCATCTA CTGCTGGTAG TCCCTGTCGA AGTTCCGGTC GTTTTCCGGT

34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
 CCTTGGCAT TTTCCGGCGC AACGACCSCA AAAAGGTATC CGAGGCGGGG

34801 CCTGACGAGC ATCACAACAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
 GGACTGCTCG TAGTGTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
 CTGTCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG

34901 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
 CGAGAGGACA AGGCTGGGAC GGCGAATGSC CTATGGACAG GCGGAAAGAG

34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
 GGAAGCCCTT CGCACCSCA AAGAGTATCG AGTCCGACAT CCATAGAGTC

35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCG
 AAGCCACATC CAGCAAGCGA GGTTGACCC GACACACGTG CTGGGGGGG

Figure 27 AK

35051 TTCAGCCCGA CC~~G~~CTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCC~~A~~AC
 AAGTCGGGCT GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG
 35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT
 GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATGTGCTTA
 35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
 ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG
 35201 CTA~~A~~CTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG
 GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC
 35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
 TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT
 35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC
 TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTGTC GTCTAATGCC
 35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGTCT
 CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA
 35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT
 CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA
 35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA
 TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT
 35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
 ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT
 35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
 AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG
 35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
 CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTACGACG
 35651 AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA
 TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT
 35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
 TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG
 35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
 CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG
 35801 GCCAGTTAAT AGTTTGC~~G~~CA AC~~G~~TTGTTGC CATTGCTACA GGCATCGTGG
 CCGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC
 35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
 ACAGTGCAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT
 35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
 AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG
 35951 CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
 GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG

Figure 2 AL

36001 TCATGGTTAT ~~GG~~AGCACTG CATAATTCTC TTAAGTGCAT GCCATC ~~TA~~
AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAGAGTGC TCATCATTTG AAAACGTTCT
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACCTA GGTCAGGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
CATTGGGTGA GCACGTGGGT TGAAGTAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTCCT

36351 ATAAGGGCGA CACGGAATG TTGAATACTC ATACTCTTCC TTTTCAATA
TATCCCGCT GTGCCCTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTG
AATAACTTCG TAAATAGTCC CAATAACAGA GACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCGA
TTACATAAAT CTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA
TTTACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA
ATTTTATCC GCATAGTGCT CCGGGAAGC AGAAGTTCTT AACCTAGGCT

PacI

~~~~~

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

*Figure 27AM*

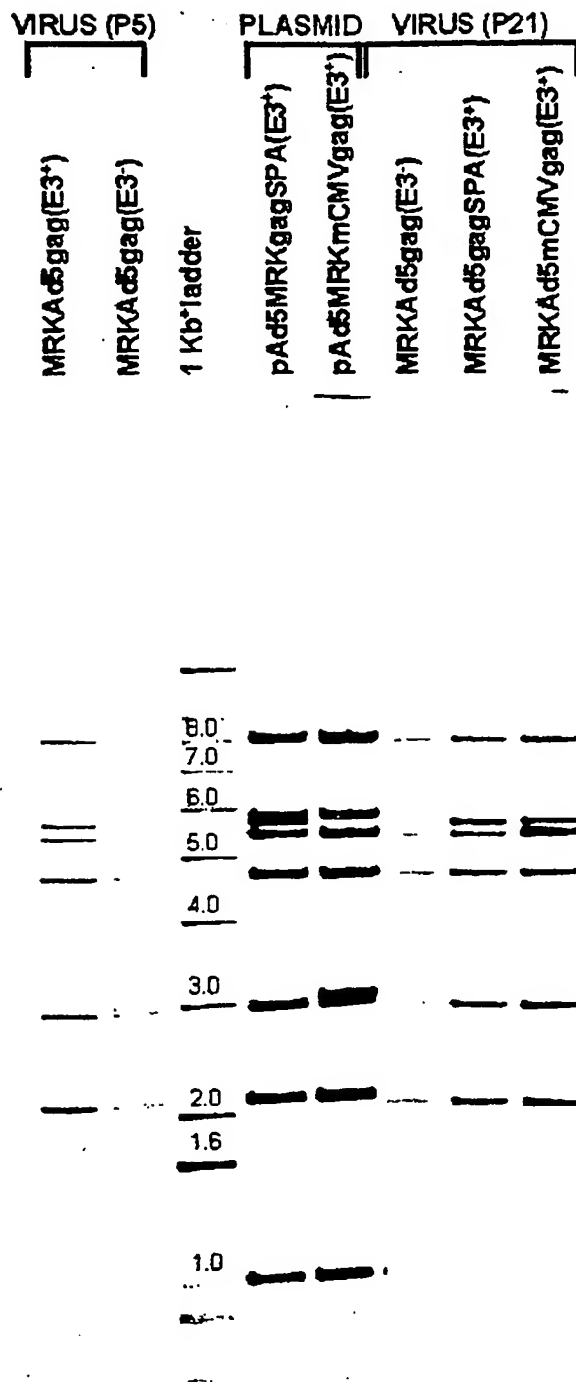


FIGURE 28

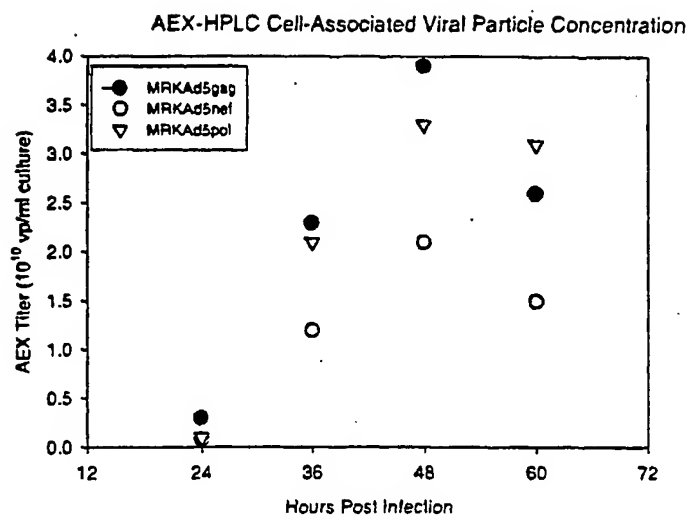


FIGURE 29A

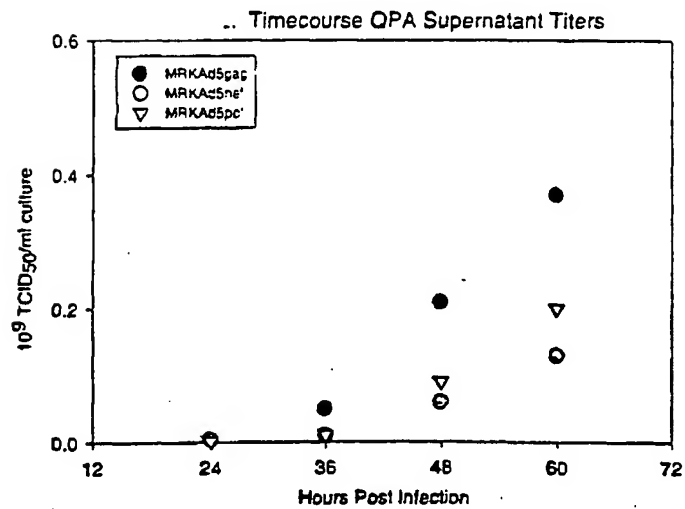


FIGURE 29B

|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga<br>Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly<br>1 5 10 15       | 48  |
| gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg<br>Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg<br>20 25 30        | 96  |
| gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag<br>Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu<br>35 40 45        | 144 |
| ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc<br>Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly<br>50 55 60        | 192 |
| tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt<br>Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys<br>65 70 75 80     | 240 |
| gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag<br>Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys<br>85 90 95        | 288 |
| att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct<br>Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala<br>100 105 110     | 336 |
| gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg<br>Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val<br>115 120 125     | 384 |
| cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc<br>Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr<br>130 135 140     | 432 |
| ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag<br>Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu<br>145 150 155 160 | 480 |
| gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac<br>Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp<br>165 170 175     | 528 |
| ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag<br>Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln<br>180 185 190     | 576 |
| atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg<br>Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu<br>195 200 205     | 624 |
| cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc<br>His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro<br>210 215 220     | 672 |
| agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att<br>Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile<br>225 230 235 240 | 720 |
| ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag<br>Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys<br>245 250 255     | 768 |

Figure 30A'



|                                                                                                                                                          |      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc<br>Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro<br>260 265 270        | 816  |
| acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac<br>Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp<br>275 280 285        | 864  |
| tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag<br>Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln<br>290 295 300        | 912  |
| gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac<br>Glu Val Lys Asn Trp Met Thr Glu Thr Leu Val Gln Asn Ala Asn<br>305 310 315 320        | 960  |
| cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg<br>Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu<br>325 330 335        | 1008 |
| gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag<br>Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys<br>340 345 350        | 1056 |
| gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc<br>Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr<br>355 360 365        | 1104 |
| atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag<br>Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys<br>370 375 380        | 1152 |
| tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc<br>Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala<br>390 395 400        | 1200 |
| ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg<br>Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met<br>405 410 415        | 1248 |
| aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc<br>Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro<br>420 425 430        | 1296 |
| tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc<br>Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro<br>435 440 445        | 1344 |
| aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc<br>Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr<br>450 455 460        | 1392 |
| ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc<br>Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala<br>465 470 475 480    | 1440 |
| tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482<br>Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37)<br>485 490 |      |

Figure 30 B

Figure 31

IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs

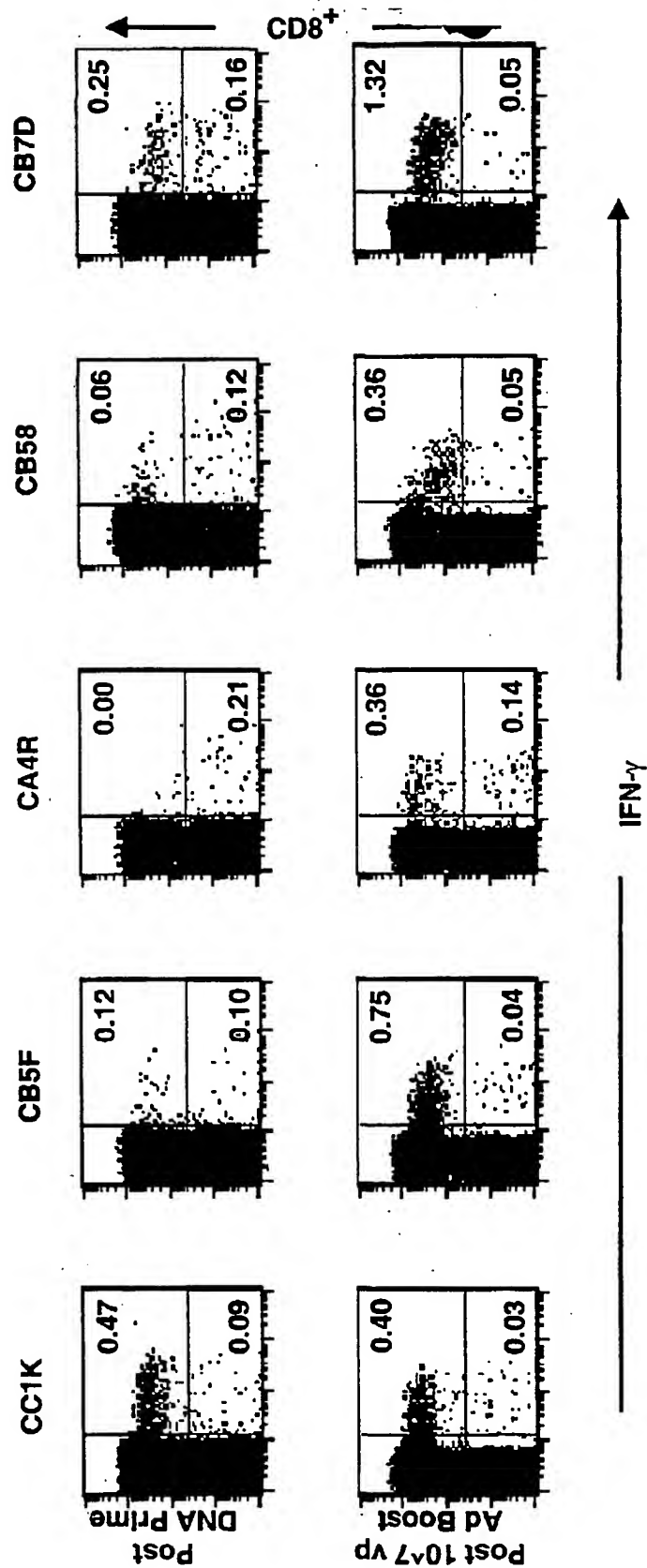


FIGURE 32

# **Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost**

## **Immunizations**

**Ad Prime/Boost**

**DNA-CRL1005 Prime/Ad Boost**

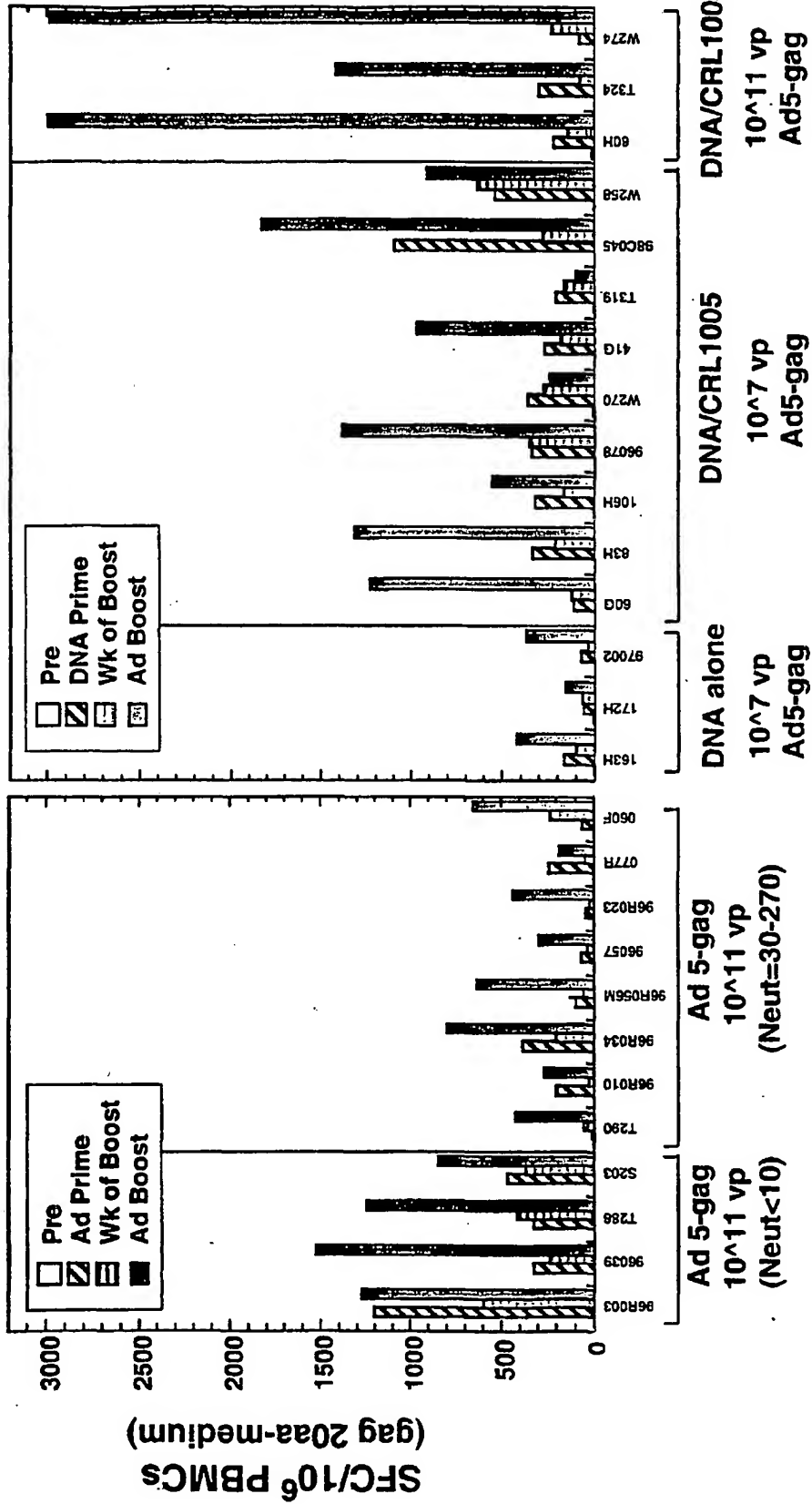


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
 CTGGGCCAGC TCCAGCCCTC CCTGCAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
 GAGAAGGCCT TCTCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA  
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC  
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT  
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
 CTGTGCTGTC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG  
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC  
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC  
 CACAAGGGCA GGCCTGGCAA CTTCTCCAG TCCAGGCCTG AGCCCACAGC CCCTCCCGAG  
 GAGTCCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC  
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCAG  
 ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
 CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC  
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
 GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCA CTTCTCTGTG  
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG  
 ACCCTTGCCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
 AAGAAGCACC AGAAGGAGCC CCCCTTCTG TGGATGGGCT ATGAGCTGCA CCCCACAAG  
 TGGACTGTGC AGCCCATTGT GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCACACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC  
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCTGTC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAATTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTCTATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAATC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA  
SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
SEQ ID NO: 39

(19) World Intellectual Property Organization  
International Bureau



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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/22080 A3



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category *    | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                              | Relevant to claim No.                                             |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| X<br>---<br>Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.                                                                                                                                          | 1-3, 8-11, 18<br>-----<br>4, 5, 13-17, 29, 30,<br>32, 34, 35, 37  |
| X<br>---<br>Y | US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.                                                                                                                                                              | 1-3, 8-11, 18<br>-----<br>4, 5, 13-17, 29, 30,<br>32, 34, 35, 37  |
| X,P           | US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.                                                                                                                                                 | 1, 9, 18                                                          |
| X<br>---<br>Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.                                                                                                                                                             | 1-3, 8, 9-11, 18<br>-----<br>4,5,13-17, 29, 30, 32,<br>34, 35, 37 |
| Y             | WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18                                                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A"

document member of the same patent family

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y          | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.                                        | 1, 9, 29, 30, 32      |
| Y          | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.                          | 1, 9, 29, 30, 32      |
| Y          | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9                  |
| Y          | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp. 115-22, see abstract.                                                                                                   | 16                    |
| Y          | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.                                                                                                                                     | 1, 9                  |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 31  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
This claim could not be searched because applicant did not provide a CRF.
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all <sup>7</sup>searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

|    |                   |                                                                                                                                                                                                                                                                                                         |
|----|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                   | and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.                                                                                                                  |
| 14 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.                  |
| 15 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.                  |
| 16 | 57-61             | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.                                                                                                                                                     |
| 17 | 62, 65, 66        | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.                                                                                                                                                        |
| 18 | 63, 64            | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                     |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.    |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.   |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.   |
| 22 | 67-70, 72, ... 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.   |
| 23 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.  |
| 24 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1. |
| 25 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1. |
| 26 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1. |
| 27 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.                  |
| 28 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.                 |
| 29 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type                                                                                                                |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

|    |                   |                                                                                                                                                                                                                                                                                                         |
|----|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                   | and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.                                                                                                                  |
| 14 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.                  |
| 15 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.                  |
| 16 | 57-61             | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.                                                                                                                                                     |
| 17 | 62, 65, 66        | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.                                                                                                                                                        |
| 18 | 63, 64            | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                     |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.    |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.   |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.   |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.   |
| 23 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.  |
| 24 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1. |
| 25 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1. |
| 26 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1. |
| 27 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.                  |
| 28 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.                 |
| 29 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type                                                                                                                |

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International application No.

PCT/US01/28861

|    |             |                                                                                                                                                                                                                                                                                         |
|----|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |             | adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.                                                                                                                                                                                          |
| 30 | 74          | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. |
| 31 | 76-80       | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.                                                                                                                                     |
| 32 | 81, 84, 85  | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.                                                                                                                                              |
| 33 | 82, 83      | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to administering a DNA plasmid vaccine.</u>                                                                                    |
| 34 | 86a         | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.                                                                                                                                                 |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.                                                                                                                                                 |
| 36 | 86c, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .                                                                            |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .                                                                            |
| 38 | 86e, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .                                                                            |
| 39 | 86f, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.                                                                                                                                   |
| 40 | 86g, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.                                                                                                                                                              |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.                                                                                                                                                             |
| 42 | 86i, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.                                                                                                                                                              |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.                                                                                                                                                        |
| 44 | 86k, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 46 | 86m, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 47 | 86n, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 48 | 86o, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1 - Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/022080 A3



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category *  | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                             | Relevant to claim No.                                   |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| X<br>—<br>Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.                                                                                                                                         | 1-3, 8-11, 18<br>—<br>4, 5, 13-17, 29-32, 34, 35, 37    |
| X<br>—<br>Y | US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.                                                                                                                                                             | 1-3, 8-11, 18<br>—<br>4, 5, 13-17, 29-32, 34, 35, 37    |
| X,P         | US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.                                                                                                                                                 | 1, 9, 18                                                |
| X<br>—<br>Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.                                                                                                                                                            | 1-3, 8, 9-11, 18<br>—<br>4, 5, 13-17, 29-32, 34, 35, 37 |
| Y           | WANG et al. The use of an E1-deleted, replication-defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18                                        |

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

| Special categories of cited documents:                                                                                                                                  |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| "E" earlier application or patent published on or after the international filing date                                                                                   | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means                                                                                            | "&" document member of the same patent family                                                                                                                                                                                                    |
| "P" document published prior to the international filing date but later than the priority date claimed                                                                  |                                                                                                                                                                                                                                                  |

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

19 AUG 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y          | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.                                        | 1, 9, 29-32           |
| Y          | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.                          | 1, 9, 29-32           |
| Y          | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9                  |
| Y          | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp. 115-22, see abstract.                                                                                                   | 16                    |
| Y          | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.                                                                                                                                     | 1, 9                  |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest

☐  
☐

- The additional search fees were accompanied by the applicant's protest.  
No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

| Group | Claims                                       |                                                                                                                                                                                                                                                                                                                                                                                             |
|-------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1     | 1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein (SEQ ID NO: 29)</u> inserted in the <u>parallel orientation</u> of E1. In addition the vector contains a promoter and a polyadenylation signal. |
| 2     | 6, 7, 36                                     | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1 and ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).                                                                                                                           |
| 3     | 12, 33                                       | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the <u>antiparallel orientation</u> of E1.                                                                                                 |
| 4     | 19-23, 38-42                                 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.                                                                                                                                                                                                                                         |
| 5     | 24, 27, 28, 43, 46, 47                       | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.                                                                                                                                                                                                                                            |
| 6     | 25, 26, 44, 45                               | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in <u>addition to</u> administering a DNA plasmid vaccine.                                                                                                                                                                                  |
| 7     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the <u>parallel orientation</u> of E1.                                                                           |
| 8     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the <u>parallel orientation</u> of E1.                                                                           |
| 9     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the <u>parallel orientation</u> of E1.                                                                           |
| 10    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the <u>antiparallel orientation</u> of E1.                                                                         |
| 11    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the <u>antiparallel orientation</u> of E1.                                                                         |
| 12    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the <u>antiparallel orientation</u> of E1.                                                                         |
| 13    | 55                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u>                                                                                                                                                                                                                                                                                              |

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|    |                   |                                                                                                                                                                                                                                                                                                         |
|----|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                   | and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.                                                                                                                  |
| 14 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.                  |
| 15 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.                  |
| 16 | 57-61             | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.                                                                                                                                                     |
| 17 | 62, 65, 66        | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.                                                                                                                                                        |
| 18 | 63, 64            | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                     |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.    |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.   |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.   |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.   |
| 23 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.  |
| 24 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1. |
| 25 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1. |
| 26 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1. |
| 27 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.                  |
| 28 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.                 |
| 29 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type                                                                                                                |

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|    |             |                                                                                                                                                                                                                                                                                         |
|----|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |             | adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.                                                                                                                                                                                          |
| 30 | 74          | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. |
| 31 | 76-80       | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.                                                                                                                                     |
| 32 | 81, 84, 85  | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.                                                                                                                                              |
| 33 | 82, 83      | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to</u> administering a DNA plasmid vaccine.                                                                                    |
| 34 | 86a         | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.                                                                                                                                                 |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.                                                                                                                                                 |
| 36 | 86c, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .                                                                            |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .                                                                            |
| 38 | 86e, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .                                                                            |
| 39 | 86f, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.                                                                                                                                   |
| 40 | 86g, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.                                                                                                                                                              |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.                                                                                                                                                             |
| 42 | 86i, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.                                                                                                                                                              |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.                                                                                                                                                        |
| 44 | 86k, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 46 | 86m, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 47 | 86n, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 48 | 86o, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erdi et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

**Continuation of B. FIELDS SEARCHED Item 3:**

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

CORRECTED VERSION

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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

TITLE OF THE INVENTION  
ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING  
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replication-defective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral-expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1 Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus  
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes  
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where  
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus  
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to  
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

#### SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not  
5 limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining  
10 the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

15 The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based  
20 adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution  
25 procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to  
30 about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the  
35 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published



January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine  
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced  
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in  
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use  
20 in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or  
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-  
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1  
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene  
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral  
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested  
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6<sup>®</sup> cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material  
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual  
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,  
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to  
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response  
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine  
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then  
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In  
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5       The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not  
10       limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen  
15       with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of  
20       such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

      The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be  
25       ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)  
30       within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second  
35       harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair  
20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a  
25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective  
immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV  
30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a  
35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to — highly active antiretroviral therapy —.

"first generation" vectors are characterized as being replication-defective.

- 5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

- 10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

- 15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

- "Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.
- 20

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

- 25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

- "Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.
- 30

- "Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.
- 35

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning



site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHPA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHPA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*II site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.)" shuttle mentioned above which contains the LA pol gene in the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises  
10 codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns  
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHPA(s)", also referred to herein as  
20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

#### BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5        Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

      Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

15        Figure 8A shows the experiment designed to test the effect of transgene orientation.

      Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20        Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

      Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

      Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

      Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

      Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5        Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed  
10        herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences  
15        through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate  
20        consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding  
25        sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as  
30        underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174  
35        and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5        Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10       Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15       Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20       Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25       Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30       Figure 31 shows the intracellular  $\gamma$ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- $\gamma$ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and  $\gamma$ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ $\gamma$ IFN+ and CD4+ $\gamma$ IFN+, respectively.

35       Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

## DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6<sup>®</sup> cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred



for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration  
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include  
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef  
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this  
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses  
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression  
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can  
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a  
5 nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of  
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,  
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a  
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and  
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or  
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.  
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may



include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with

5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral  
10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a  
15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.  
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino  
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most  
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells  
5 for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully  
10 transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of  
15 this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

20 Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient  
25 to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed  
30 *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin  
35 resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors  
5 not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol,  
10 pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were  
15 harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby  
20 incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>,  
25 from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above  
30 description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be  
35 used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as  
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM  $MgCl_2$ ; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably  
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used to make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM  $MgCl_2$ , 0.005% polysorbate 80 at pH 8.0. This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.  
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene  
20 product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also  
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine  
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile  
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

#### EXAMPLE 1

##### Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *BglII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglII*. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *BglII* digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *BglII* site. Colonies were screened using *SmaI* restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTGTGTG  
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

## EXAMPLE 2

### Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

| Plasmid                              | $\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$ |
|--------------------------------------|-------------------------------------------------------------------------|
| HIVFL-gagPR9901 <sup>a</sup>         | 10.8                                                                    |
| pV1Jns-hCMV-FLgag-bGHpA <sup>b</sup> | 16.6                                                                    |
| pV1Jns-hCMV-FLgag-SPA <sup>b,c</sup> | 12.0                                                                    |

<sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5 <sup>b</sup> New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

### 10 EXAMPLE 3

#### Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above  
 15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which  
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20  $\mu\text{g}$  and 200  $\mu\text{g}$ .



## EXAMPLE 4

**Table 3:** HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

| DNA <sup>a</sup><br>Promoter/terminator | Dose,<br>ug <sup>b</sup> | Anti-p24 Titers<br>(3 Wk PD1) <sup>c</sup> |      |      | SFC/10 <sup>6</sup> Cells<br>(4 Wk PD1) <sup>d</sup> |            |        |
|-----------------------------------------|--------------------------|--------------------------------------------|------|------|------------------------------------------------------|------------|--------|
|                                         |                          | GMT                                        | +SE  | -SE  | Media                                                | gag197-205 | p24    |
| HIVFL-gagPR9901<br>(GMP grade)          | 200                      | 12800                                      | 4652 | 3412 | 2(2)                                                 | 129(19)    | 30(11) |
|                                         | 20                       | 5572                                       | 1574 | 1227 | 0                                                    | 56(9)      | 25(6)  |
| pV1Jns-hCMV-<br>FL-gag-bGHpA            | 200                      | 11143                                      | 2831 | 2257 | 0                                                    | 98(5)      | 12(6)  |
|                                         | 20                       | 7352                                       | 2808 | 2032 | 0                                                    | 73(9)      | 11(6)  |
| pV1Jns-hCMV-<br>FL-gag-SPA              | 200                      | 16890                                      | 5815 | 4326 | 1(1)                                                 | 94(4)      | 26(7)  |
|                                         | 20                       | 5971                                       | 5361 | 2825 | 0                                                    | 85(17)     | 38(10) |
| Naïve                                   | 0                        | 123                                        | 50   | 36   | 0                                                    | 0          | 0      |

<sup>a</sup>In PBS<sup>b</sup>i.m. Injections into both quads, 50 µL per quad<sup>c</sup>n=10; GMT, geometric mean titer; SE, standard. error<sup>d</sup>n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

## Construction of the Modified Shuttle Vector -"MRKpdeIE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
  - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

## EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac*I and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*I linearized pAdHVO (E3- adenovector) or *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I, *Bam*HI, *Xho*I, *Eco*RV, *Hind*III, *Sal*I, and *Bgl*II sites. This MCS was replaced with a new MCS containing *Not*I, *Cla*I, *Eco*RV and *Asc*I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

## EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*I to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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#### EXAMPLE 7

##### Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*I to remove the vector backbone) and then labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

### EXAMPLE 8

5 Construction of the new shuttle vector containing modified gag transgene –  
“MRKpdelE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHPA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeI1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeI1 shuttle vector.

### EXAMPLE 9

### Construction of the MRK FG-Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*I. The reaction mixture was digested with *Bsf*ZI71. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*I overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH<sub>2</sub>O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

## EXAMPLE 10

### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

## EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *PacI* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [<sup>33</sup>P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *PacI/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

## EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.



Table 4:  
Amplification Ratios Based on AEX and QPA Analysis of  
Virus Amplification from Passage 3 to Passage 4.

| Ad gag construct       | Amplification Ratio |
|------------------------|---------------------|
| MRKAd5gag              | 470                 |
| HCMV-Flgag-bGHpA [E3-] | 115                 |
| HCMV-Flgag-SPA [E3+]   | 320                 |
| mCMV-FLgag-bGHpA [E3+] | 420                 |
| Original construct *   | 40 - 50             |

\* This estimation is based on the clinical lot growth characteristics at Passage 12.

### EXAMPLE 13

#### Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5           Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10   Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

### MRKAd5gag rep1

|     | Xv (10 <sup>6</sup> cells/ml),<br>Infection | Viability (%)<br>Harvest | Harvest Time<br>h.p.i. | Cell Passage<br>Number | Titer<br>10 <sup>6</sup> vp/ml culture | Titer<br>10 <sup>6</sup> vp/cell | QPA<br>10 <sup>6</sup> TCID <sub>50</sub> /ml | Ratio<br>AEX:QPA | Amplification<br>Ratio | AEX<br>Internal Control |
|-----|---------------------------------------------|--------------------------|------------------------|------------------------|----------------------------------------|----------------------------------|-----------------------------------------------|------------------|------------------------|-------------------------|
| P4  | 1.49, 81%                                   | 0.58, 50%                | 44                     | 46                     | 8.7                                    | 6.9                              | 1.72                                          | 50               | 470<br>(MOI = 125)     |                         |
| P5  | 1.38, 93%                                   | 0.66, 47%                | 48                     | 49                     | 6.7                                    | 4.9                              | 1.38                                          | 49               | 170                    |                         |
| P6  | 1.04, 94%                                   | 0.68, 77%                | 47                     | 48                     | 5.6                                    | 8.6                              | 1.42                                          | 41               | 200                    |                         |
| P7  | 1.50, 94%                                   | 0.88, 61%                | 49.5                   | 50                     | 3.9                                    | 1.4                              | 0.87                                          | 40               | 50                     |                         |
| P7  | 1.09, 97%                                   | 0.78, 59%                | 50                     | 52                     | 6.2                                    | 4.7                              | 1.70                                          | 81               | 170                    |                         |
| P8  | 1.03, 94%                                   | 0.86, 64%                | 47.5                   | 64                     | 9.0                                    | 6.7                              | 1.10                                          | 82               | 310                    |                         |
| P9  | 0.89, 95%                                   | 0.99, 73%                | 47.5                   | 56                     | 4.4                                    | 4.5                              | 1.03                                          | 43               | 175                    | 3.12                    |
| P10 | 1.09, 91%                                   | 1.08, 66%                | 47.5                   | 58                     | 3.0                                    | 2.5                              | 1.16                                          | 26               | 100                    | 2.84                    |
| P11 | 1.18, 88%                                   | 0.88, 65%                | 47                     | 60                     | 3.8                                    | 3.0                              | 1.15                                          | 31               | 110                    | 2.70                    |
| P12 | 0.88, 91%                                   | 0.85, 63%                | 47.5                   | 47                     | 5.4                                    | 5.5                              | 1.20                                          | 45               | 200                    | 2.60                    |
| P13 | 1.00, 88%                                   | 0.70, 67%                | 48                     | 49                     | 5.8                                    | 5.8                              | 1.11                                          | 52               | 210                    | 2.70                    |
| P14 | 1.84, 92%                                   | 0.88, 67%                | 46                     | 53                     | 8.6                                    | 4.4                              |                                               |                  | 160                    | 3.18                    |
| P15 | 0.97, 96%                                   | 0.64, 66%                | 47                     | 47                     | 6.9                                    | 7.1                              |                                               |                  | 250                    | 3.27                    |
|     |                                             |                          |                        |                        |                                        |                                  |                                               |                  |                        | 3.12                    |
|     |                                             |                          |                        |                        |                                        |                                  |                                               |                  |                        | 2.91                    |

**Table 5B:** Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

### MRKHVE3

|     | Xv (10 <sup>6</sup> cells/ml),<br>Infection | Viability (%)<br>Harvest | Harvest Time<br>h.p.i. | Cell Passage<br>Number | Titer<br>10 <sup>6</sup> vp/ml culture | Titer<br>10 <sup>6</sup> vp/cell | QPA<br>10 <sup>6</sup> TCID <sub>50</sub> /ml | Ratio<br>AEX:QPA | Amplification<br>Ratio | AEX<br>Internal Control |
|-----|---------------------------------------------|--------------------------|------------------------|------------------------|----------------------------------------|----------------------------------|-----------------------------------------------|------------------|------------------------|-------------------------|
| P4  | 1.10, 87%                                   | 1.28, 76%                | 49                     | 54                     | 4.1                                    | 3.8                              | 1.70                                          | 25               | 300<br>(MOI = 125)     |                         |
| P5  | 0.82, 89%                                   | 1.18, 77%                | 47                     | 48                     | 4.3                                    | 4.7                              | 1.24                                          | 35               | 170                    |                         |
| P6  | 1.55, 88%                                   | 1.26, 76%                | 49.5                   | 60                     | 1.2                                    | 0.8                              | 0.58                                          | 21               | 30                     |                         |
| P6  | 1.09, 97%                                   | 1.11, 81%                | 49                     | 52                     | 4.0                                    | 2.5                              | 1.16                                          | 34               | 130                    |                         |
| P7  | 1.17, 91%                                   | 1.22, 81%                | 47.5                   | 54                     | 3.7                                    | 3.2                              | 0.50                                          | 74               | 110                    |                         |
| P8  | 0.98, 88%                                   | 1.41, 63%                | 48                     | 56                     | 2.1                                    | 2.1                              | 0.47                                          | 45               | 75                     | 3.12                    |
| P9  | 1.20, 89%                                   | 1.26, 81%                | 47.5                   | 58                     | 0.8                                    | 0.7                              | 0.29                                          | 28               | 25                     | 2.84                    |
| P10 | 0.95, 82%                                   | 1.55, 65%                | 47                     | 50                     | 2.3                                    | 2.3                              | 0.43                                          | 53               | 80                     | 2.70                    |
| P11 | 1.07, 96%                                   | 1.25, 63%                | 48                     | 47                     | 2.7                                    | 2.5                              | 0.41                                          | 66               | 90                     | 2.70                    |
| P12 | 0.80, 91%                                   | 1.14, 60%                | 48.5                   | 49                     | 6.9                                    | 7.4                              | 0.48                                          | 123              | 250                    | 2.68                    |
| P13 | 1.86, 95%                                   | 1.14, 66%                | 45.5                   | 53                     | 6.8                                    | 3.0                              |                                               |                  | 110                    | 2.85                    |
| P14 | 0.87, 96%                                   | 1.00, 88%                | 48.5                   | 47                     | 9.4                                    | 8.7                              |                                               |                  | 350                    | 3.27                    |
| P15 | 0.87, 89%                                   | 0.87, 59%                | 49.5                   | 49                     | 6.3                                    | 6.1                              |                                               |                  | 218                    | 3.12                    |
|     |                                             |                          |                        |                        |                                        |                                  |                                               |                  |                        | 2.91                    |
|     |                                             |                          |                        |                        |                                        |                                  |                                               |                  |                        | 2.78                    |
|     |                                             |                          |                        |                        |                                        |                                  |                                               |                  |                        | 2.52                    |

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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### MRKAd5gag(E3-)

|     | Xv (10 <sup>6</sup> cells/ml) | Viability (%) | Harvest Time | Cell Passage | Titer                         | Titer                   | QPA                                    | Ratio   | Amplification | AEX              |
|-----|-------------------------------|---------------|--------------|--------------|-------------------------------|-------------------------|----------------------------------------|---------|---------------|------------------|
|     | Infection                     | Harvest       | h.p.i.       | Number       | 10 <sup>4</sup> vp/ml culture | 10 <sup>4</sup> vp/cell | 10 <sup>4</sup> TCID <sub>50</sub> /ml | AEX:QPA | Ratio         | Internal Control |
| P4  | 1.82, 77%                     | 1.12, 82%     | 47.5         | 46           | 2.0                           | 1.2                     | 0.92                                   | 20      | 100           |                  |
| P5  | 1.16, 82%                     | 0.82, 43%     | 49           | 49           | 3.3                           | 2.9                     | 0.99                                   | 34      | 100           |                  |
| P6  | 1.71, 86%                     | 0.20, 10%     | 49           | 50           | 4.7                           | 2.7                     | 1.70                                   | 26      | 100           |                  |
| P8  | 1.08, 97%                     | 0.63, 64%     | 49.5         | 52           | 5.4                           | 5.0                     | 1.76                                   | 31      | 180           |                  |
| P7  | 1.17, 91%                     | 0.98, 72%     | 47.50        | 54           | 7.1                           | 6.1                     | 0.67                                   | 106     | 220           |                  |
| P8  | 0.98, 88%                     | 0.77, 48%     | 48           | 56           | 3.1                           | 3.2                     | 0.66                                   | 47      | 115           | 3.12             |
| P9  | 1.20, 89%                     | 1.03, 72%     | 48           | 58           | 1.8                           | 1.5                     | 0.57                                   | 32      | 55            | 2.84             |
| P10 | 0.93, 82%                     | 0.80, 62%     | 48.5         | 60           | 3.2                           | 3.2                     | 0.68                                   | 47      | 115           | 2.70             |
| P11 | 1.07, 96%                     | 0.88, 70%     | 48.5         | 47           | 5.9                           | 5.6                     | 0.68                                   | 87      | 200           | 2.60             |
| P12 | 0.80, 81%                     | 0.87, 59%     | 50           | 49           | 5.1                           | 6.4                     | 0.72                                   | 71      | 230           | 2.88             |
| P13 | 1.96, 95%                     | 0.91, 59%     | 45.5         | 53           | 7.4                           | 3.8                     |                                        |         | 135           | 3.18             |
| P14 | 0.97, 96%                     | 0.81, 74%     | 48           | 47           | 6.8                           | 7.0                     |                                        |         | 250           | 3.28             |
| P15 | 0.87, 89%                     | 0.84, 66%     | 49           | 49           | 4.8                           | 5.5                     |                                        |         | 196           | 3.27             |
|     |                               |               |              |              |                               |                         |                                        |         |               | 2.78             |
|     |                               |               |              |              |                               |                         |                                        |         |               | 2.52             |

### EXAMPLE 14

#### Gag Expression Analysis of the Novel Constructs

*In vitro* gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

### EXAMPLE 15

#### Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag ( $10^7$  and  $10^9$  vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

| Viral Vectors <sup>a</sup>       | $\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$ |
|----------------------------------|------------------------------------------------------------------------------|
| MRKAd5gag <sup>b</sup>           | 1.40                                                                         |
| Clinical lot Ad5gag <sup>c</sup> | 1.28                                                                         |
| Research lot Ad5gag <sup>d</sup> | 1.32                                                                         |
| MCMVFL-gagbGHpA <sup>e</sup>     | 0.42                                                                         |

<sup>a</sup>  $A_{260\text{nm}}$  absorbance readings taken for viral particle determinations.

<sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>d</sup> Research Ad5FLgag lot# 6399

<sup>e</sup> mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

**Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).**

| Group ID | Vaccine                                         | Dose (vp)       | GMT    | SE upper | SE lower |
|----------|-------------------------------------------------|-----------------|--------|----------|----------|
| 1        | <sup>a</sup> MRKAd5gag                          | 10 <sup>7</sup> | 25600  | 5877     | 4780     |
| 2        | "                                               | 10 <sup>9</sup> | 409600 | 94028    | 76473    |
| 3        | hCMV FL-gag bGHpA [E3-] →                       | 10 <sup>7</sup> | 7352   | 2077     | 1620     |
| 4        | "                                               | 10 <sup>9</sup> | 235253 | 59767    | 47659    |
| 5        | hCMV FL-gag SPA [E3+] →                         | 10 <sup>7</sup> | 12800  | 9905     | 236      |
| 6        | "                                               | 10 <sup>9</sup> | 310419 | 99181    | 75165    |
| 7        | <sup>b</sup> mCMV FL-gag bGHpA [E3+] →          | 10 <sup>7</sup> | 44572  | 23504    | 15389    |
| 8        | "                                               | 10 <sup>9</sup> | 941014 | 239068   | 190636   |
| 9        | <sup>c</sup> hCMV FL-gag bGHpA [E3-] ←          | 10 <sup>7</sup> | 3676   | 934      | 745      |
| 10       | "                                               | 10 <sup>9</sup> | 117627 | 17491    | 15227    |
| 11       | research lot hCMV intronA FL-gag bGHpA [E3-] <- | 10 <sup>6</sup> | 528    | 262      | 175      |
| 12       | "                                               | 10 <sup>7</sup> | 14703  | 5274     | 3882     |
| 13       | "                                               | 10 <sup>8</sup> | 58813  | 14942    | 11915    |
| 14       | "                                               | 10 <sup>9</sup> | 204800 | 53232    | 42250    |
| 15       | clinical lot hCMVintronA FL-gag bGHpA [E3-] <-  | 10 <sup>6</sup> | 230    | 82       | 61       |
| 16       | "                                               | 10 <sup>7</sup> | 4222   | 3405     | 1138     |
| 17       | "                                               | 10 <sup>8</sup> | 19401  | 3939     | 3274     |
| 18       | "                                               | 10 <sup>9</sup> | 89144  | 25187    | 19639    |
| 19       | Naïve                                           | none            | 93     | 7        | 6        |

\*2x50 µL i.m. (quad) injections/animal

†Ls: Youil, Chen, Gasimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

<sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

<sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10<sup>6</sup> dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

### EXAMPLE 16

#### Comparison of Humoral and Cellular Responses Towards the Original Ad-gag

#### Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10<sup>11</sup> vp and 10<sup>9</sup> vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-  
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assmumarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after
- 5 CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

| Vaccine                                                                     | Pre | Wk 4 | Wk 8 | Wk 12 | Wk 16 | Wk 20 | Wk 25 | Wk 28 |
|-----------------------------------------------------------------------------|-----|------|------|-------|-------|-------|-------|-------|
| MR KAd5gag <sup>0</sup> , 10 <sup>11</sup> vp                               |     |      |      |       |       |       |       |       |
| 97N010                                                                      | <10 | 118  | 5528 | 11523 | 7062  | 21997 | ND    | 51593 |
| 97N116                                                                      | <10 | 62   | 772  | 1447  | 1562  | 2174  | ND    | 20029 |
| 98X007                                                                      | <10 | 66   | 3353 | 6156  | 6845  | 3719  | ND    | 24031 |
| MR KAd5gag, 10 <sup>9</sup> vp                                              |     |      |      |       |       |       |       |       |
| 97N120                                                                      | <10 | 51   | 204  | 318   | 366   | 482   | ND    | 6550  |
| 97N144                                                                      | <10 | 18   | 118  | 274   | 706   | 888   | ND    | 7136  |
| 98X008                                                                      | <10 | 15   | 444  | 386   | 996   | 1072  | ND    | 12851 |
| Ad5gag <sup>0</sup> , Clinical Lot, 10 <sup>11</sup> vp                     |     |      |      |       |       |       |       |       |
| 97X001                                                                      | <10 | 87   | 2579 | 4718  | 7174  | 7250  | ND    | 69226 |
| 97N146                                                                      | <10 | 72   | 3604 | 7380  | 7526  | 18906 | ND    | 60283 |
| 98X009                                                                      | <10 | 78   | 4183 | 3946  | 3124  | 6956  | ND    | 26226 |
| Ad5gag, Clinical Lot, 10 <sup>9</sup> vp                                    |     |      |      |       |       |       |       |       |
| 97N020                                                                      | <10 | <10  | 143  | 371   | 390   | 1821  | ND    | 17177 |
| 97X003                                                                      | <10 | <10  | 39   | 93    | 156   | 596   | ND    | 2053  |
| 98X012                                                                      | <10 | 81   | 342  | 717   | 956   | 1558  | ND    | 11861 |
| MR KAd5gag (hCMV, bGHpA, E3+)                                               |     |      |      |       |       |       |       |       |
| <sup>0</sup> original Ad5gag vector (hCMV/Intron A, bGHpA, E3-), lot#FN0001 |     |      |      |       |       |       |       |       |
| ND, not determined                                                          |     |      |      |       |       |       |       |       |

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

| Grp # | Vaccination<br>T=0,4,25 wks               | Monkey ID    | T=4 Wk             |                    | T=6 Wk |       | T=11 Wk |       | T=16 Wk |       | T=25 Wk |       | T=28 Wk |       |
|-------|-------------------------------------------|--------------|--------------------|--------------------|--------|-------|---------|-------|---------|-------|---------|-------|---------|-------|
|       |                                           |              | Media <sup>a</sup> | Gag H <sup>b</sup> | Media  | Gag H | Media   | Gag H | Media   | Gag H | Media   | Gag H | Media   | Gag H |
| 1     | MRKAd5gag<br>10 <sup>9</sup> vp           | 97ND10       | 6                  | 89                 | 0      | 395   | 0       | 1058  | 0       | 1174  | 3       | 775   | 4       | 1074  |
|       |                                           | 97ND10(CD4-) | 4                  | 38                 |        |       | 3       | 993   |         |       | 0       | 76    | 0       | 594   |
|       |                                           | 97N116       | 1                  | 388                | 1      | 609   | 0       | 534   | 4       | 395   | 1       | 261   | 0       | 408   |
|       |                                           | 97N116(CD4-) | 11                 | 676                |        |       | 0       | 593   |         |       | 0       | 184   | 0       | 666   |
|       |                                           | 98X007       | 10                 | 579                | 0      | 1304  | 3       | 2193  | 1       | 2118  | 3       | 1588  | 0       | 2113  |
|       |                                           | 98X007(CD4-) | 20                 | 965                |        |       | 0       | 2675  |         |       | 0       | 1656  | 0       | 1278  |
| 2     | MRKAd5gag<br>10 <sup>9</sup> vp           | 97N120       | 5                  | 275                | 1      | 249   | 4       | 141   | 4       | 119   | 9       | 206   | 4       | 219   |
|       |                                           | 97N120(CD4-) | 11                 | 170                |        |       | 0       | 85    |         |       | 0       | 75    | 1       | 219   |
|       |                                           | 97N144       | 3                  | 236                | 6      | 438   | 1       | 318   | 3       | 256   | 1       | 98    | 5       | 373   |
|       |                                           | 97N144(CD4-) | 6                  | 148                |        |       | 0       | 285   |         |       | ND      | ND    | 0       | 625   |
|       |                                           | 98X008       | 4                  | 368                | 1      | 1090  | 3       | 891   | 4       | 673   | 3       | 473   | 5       | 735   |
|       |                                           | 98X008(CD4-) | 14                 | 696                |        |       | 0       | 1175  |         |       | 0       | 391   | 4       | 848   |
| 3     | Ad5gag clinical lot<br>10 <sup>9</sup> vp | 97X001       | 0                  | 281                | 1      | 485   | 0       | 817   | 0       | 1220  | 1       | 894   | 0       | 1858  |
|       |                                           | 97X001(CD4-) | 10                 | 283                |        |       | 3       | 996   |         |       | 0       | 1010  | 0       | 1123  |
|       |                                           | 97N146       | 3                  | 150                | 1      | 485   | 0       | 339   | 1       | 1272  | 3       | 1238  | 3       | 1785  |
|       |                                           | 97N146(CD4-) | 6                  | 133                |        |       | 0       | 370   |         |       | 0       | 654   | 0       | 971   |
|       |                                           | 98X009       | 0                  | 93                 | 3      | 339   | 3       | 559   | 0       | 896   | 1       | 384   | 0       | 1748  |
|       |                                           | 98X009(CD4-) | 0                  | 73                 |        |       | 0       | 333   |         |       | 0       | 225   | 0       | 644   |
| 4     | Ad5gag clinical lot<br>10 <sup>9</sup> vp | 97ND20       | 3                  | 30                 | 1      | 101   | 0       | 66    | 0       | 36    | 0       | 26    | 0       | 41    |
|       |                                           | 97ND20(CD4-) | 10                 | 29                 |        |       | 0       | 15    |         |       | 0       | 1     | 0       | 16    |
|       |                                           | 97X003       | 4                  | 68                 | 8      | 134   | 0       | 18    | 1       | 38    | 4       | 38    | 6       | 81    |
|       |                                           | 97X003(CD4-) | 9                  | 40                 |        |       | 0       | 6     |         |       | 0       | 4     | 0       | 19    |
|       |                                           | 98X012       | 5                  | 85                 | 3      | 54    | 1       | 34    | 0       | 18    | 0       | 20    | 1       | 121   |
|       |                                           | 98X012(CD4-) | 11                 | 70                 |        |       | 0       | 11    |         |       | 0       | 8     | 0       | 41    |
| 5     | Native                                    | 96R041       | 6                  | 8                  | 1      | 1     | 0       | 0     | 0       | 0     | 0       | 0     | 1       | 0     |
|       |                                           | Q53F         | 14                 | 18                 | 5      | 16    | 20      | 14    | 19      | 15    | 10      | 15    | 24      | 9     |

<sup>a</sup>Based on either 4x10<sup>6</sup> or 2x10<sup>6</sup> cells per well (depending on spot density)

<sup>b</sup>... determined

<sup>c</sup>Mock or no peptide control

<sup>d</sup>Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10<sup>9</sup> vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

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### EXAMPLE 17

#### CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

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The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based



on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

```

AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

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GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC  
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG  
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC  
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC  
5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC  
AACAAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC  
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC  
CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT  
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC  
10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCTGTG GGATGGGCTA TGAGCTGCAC  
CCCACAAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT  
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG  
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG  
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT  
15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC  
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC  
AGSATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC  
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG  
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG  
20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG  
GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT  
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG  
AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT  
GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT  
25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG  
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC  
ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC  
CACTCCAACCT GGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG  
ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC  
30 TGCTCCCTG GCATCTGGCA GCTGGACTGC ACCACCTGG AGGGCAAGGT GATCCTGGTG  
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
GAGACTGCCT ACTTCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGA GTCCATGAAC  
35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
GTGCAGATGG CTGTGTTTCT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 CAGATCACCA AGATCCAGAA CTTCAAGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ  
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID  
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg  
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly  
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu  
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which  
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to  
 deletion of the portion of the wild type sequence encoding the protease activity, a  
 30 combination of active site residue mutations are introduced which are deleterious to  
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present  
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein  
 the construct is devoid of DNA sequences encoding any PR activity, as well as  
 containing a mutation(s) which at least partially, and preferably substantially,  
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part  
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

|    | <u>wt aa</u> | <u>aa residue</u> | <u>mutant aa</u> | <u>enzyme function</u> |
|----|--------------|-------------------|------------------|------------------------|
|    | Asp          | 112               | Ala              | RT                     |
|    | Asp          | 187               | Ala              | RT                     |
| 30 | Asp          | 188               | Ala              | RT                     |
|    | Asp          | 445               | Ala              | RNase H                |
|    | Glu          | 480               | Ala              | RNase H                |
|    | Asp          | 500               | Ala              | RNase H                |
|    | Asp          | 626               | Ala              | IN                     |
| 35 | Asp          | 678               | Ala              | IN                     |
|    | Glu          | 714               | Ala              | IN                     |

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

5 AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC  
 ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG  
 GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC  
 10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG  
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC  
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC  
 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTEACCAT CCCCTCCATC  
 AACAAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC  
 15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC  
 CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT  
 TGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC  
 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC  
 CCCGACAAGT GGACTGTGCA GCCCATTTGTG CTGCCCTGAGA AGGACTCCTG GACTGTGAAT  
 20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG  
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG  
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT  
 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC  
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC  
 25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC  
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG  
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG  
 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG  
 GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT  
 30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG  
 AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT  
 GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT  
 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG  
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC  
 35 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC  
 CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC  
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTAT CCACAATTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC  
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 10 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACCTC  
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID  
 NO:3) .

15 In order to produce the IA-pol-based adenoviral vaccines of the present  
 invention, inactivation of the enzymatic functions was achieved by replacing a total of  
 nine active site residues from the enzyme subunits with alanine side-chains. As  
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,  
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues  
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*  
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,  
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this  
 IA Pol construct), with each residue being substituted for an Ala residue, respectively  
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-  
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase  
 function was abolished through three mutations at Asp626, Asp678 and Glu714.  
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,  
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-  
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.  
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and  
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly  
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala  
Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala  
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly



Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu  
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations  
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based  
 adenoviral HIV vaccine of the present invention, either when administered alone or in  
 a combined modality regime and/or a prime-boost regimen. For example, it may be  
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,  
 RNase-H, and integrase coding regions while still abolishing these enzymatic  
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID  
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also  
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1  
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal  
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide  
 such as is found in highly expressed mammalian proteins such as immunoglobulin  
 leader peptides. Any functional leader peptide may be tested for efficacy. However,  
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown  
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein  
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,  
 preferably a leader peptide from human tPA. In other words, a codon optimized  
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide  
 at the amino terminal portion of the protein, which may effect cellular trafficking and  
 hence, immunogenicity of the expressed protein within the host cell. As noted in  
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention  
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region ( herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT  
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCCTCAC  
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT  
35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC  
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCAT TGTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGG CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT  
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCAT TGTGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA  
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 15 GGAGGTGAAC ATTGTGACTG ACTCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGTCTCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA  
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGACAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT  
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ  
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:  
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser  
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr  
Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala  
Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr  
Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu  
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe  
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6) .

The present invention also relates to a codon optimized HIV-1 Pol mutant  
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)  
 which comprises a leader peptide at the amino terminal portion of the protein, which  
 may effect cellular trafficking and hence, immunogenicity of the expressed protein  
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in  
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a  
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,  
 any such leader peptide-based HIV-1 pol mutant construct may include but is not  
 limited to a mutated DNA molecule which effectively alters the catalytic activity of  
 the RT, RNase and/or IN region of the expressed protein, resulting in at least  
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN  
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a  
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the  
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An  
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at  
 least one point mutation which alters the active site and catalytic activity within the  
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially  
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed  
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open  
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
 CTTCTGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAATCT CCAAGATTGG  
 CCCCAGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT  
 20 GGGGGATGCC TACTTCTCTG TGCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC  
 CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
 GGGCTGGAAG GGCTCCCTCG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT  
 CAGGAAGCAG AACCTTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC  
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG  
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCAT TGTCTGCCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT  
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCAT TGTGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 5 GTACCTGGCC TGGGTGCCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA  
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT  
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 ACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile  
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu  
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe



Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8) .

### EXAMPLE 18

#### 10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed  
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef  
 20 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein  
 30 described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfr1 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

10 GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
15 GCTTCCCCGT GAGGCCCCAG GTGCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
TGTCCTCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
AATACCCCCGG CCCCAGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC  
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGCACT  
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
AAAGCCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),  
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);  
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),  
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian  
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby  
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.  
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating  
methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides  
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid  
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine  
vector. The 216 amino acid HIV-1 Nef (jfr1) protein is disclosed herein as SEQ ID  
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the  
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2  
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions  
 have been elucidated, it has become clear that correct trafficking of Nef to the inner  
 plasma membrane promotes viral replication by altering the host intracellular  
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the  
 20 infectivity of progeny viral particles. In one aspect of the invention regarding  
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the  
 adenovirus vector of the present invention is modified to contain a nucleotide  
 sequence which encodes a heterologous leader peptide such that the amino terminal  
 region of the expressed protein will contain the leader peptide. The diversity of  
 25 function that typifies eukaryotic cells depends upon the structural differentiation of  
 their membrane boundaries. To generate and maintain these structures, proteins must  
 be transported from their site of synthesis in the endoplasmic reticulum to  
 predetermined destinations throughout the cell. This requires that the trafficking  
 proteins display sorting signals that are recognized by the molecular machinery  
 30 responsible for route selection located at the access points to the main trafficking  
 pathways. Sorting decisions for most proteins need to be made only once as they  
 traverse their biosynthetic pathways since their final destination, the cellular location  
 at which they perform their function, becomes their permanent residence.  
 Maintenance of intracellular integrity depends in part on the selective sorting and  
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs  
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRG LCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

```

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG
GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAAC TGCCTGCTGC ACCCATGTG
CCAGCACGGC ATCAGGAGC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT
GGCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11) .

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfr1) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
 Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfr1 isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfr1 nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
 ACACCCCCGG CCCCAGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCACC  
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT  
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val  
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA  
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.  
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which  
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue  
 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174  
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

5 CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT  
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG  
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG  
10 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC  
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC  
CGTGAGGCCC CAGGTGCCCC TGAGGCCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA  
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT  
15 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC  
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG  
GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC  
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTTCG ACTCCAAGCT  
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC  
15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175



and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

## EXAMPLE 19

### MRKAd5Pol Construction and Virus Rescue

*Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*I site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using  
 5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its  
 10 isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Clal* digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Clal*. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA  
 15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-  
 25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing  
 30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

## EXAMPLE 20

### MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl11* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl11* releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdeIE1+CMVmin+BGHPA(str.) shuttle vector at the *Bgl11* site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca1*. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHPA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHPA(s) was digested with restriction enzymes *Pac1* and *Bst1107 I* (or its isoschizomer, *BstZ107 I*) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla1* digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHPA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

*Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac1* (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac1* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq -60^{\circ}\text{C}$ . This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

### EXAMPLE 21

#### Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

10 The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

*Bgl* II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

#### EXAMPLE 22

##### 5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene  
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla*I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently  
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

#### EXAMPLE 23

##### Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (*Bam*HI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (*Bam*HI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).  
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complete coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca*I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac*I and *Bst*Z110I and cloned into the E3+ MRKAd5 adenovector via bacterial  
30 homologous recombination techniques.

#### EXAMPLE 24

##### Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c  
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either  $10^7$  vp and  $10^9$  vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were  
5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50  $\mu$ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following  
10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were  
15 collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively.

*Non-human Primate immunization* - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IAPol (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp dose; and (2) MRKAd5hCMV-IAPol (E3-) at either  
20 10<sup>9</sup> vp and 10<sup>11</sup> vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0)  
25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

*Murine anti-RT and anti-nef ELISA* - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 RT protein  
30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200  $\mu$ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was  
35 performed followed by 4-fold serial dilution. 100- $\mu$ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by  
 5 adding 100  $\mu$ L of 0.5M H<sub>2</sub>SO<sub>4</sub> per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

*Non-human primate and murine ELISpot assays* - The enzyme-linked  
 10 immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF $\gamma$ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at  $5 \times 10^6$ /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL  
 15 streptomycin, 10 mM Hepes, 50 uM  $\beta$ -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen aqueous plates (Millipore, France) were coated with 100  $\mu$ L/well of either 5  $\mu$ g/mL purified rat anti-mouse IFN- $\gamma$  IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or  
 20 15  $\mu$ g/mL mouse anti-human IFN- $\gamma$  IgG<sub>2a</sub> (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200  $\mu$ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50  $\mu$ L of cell samples ( $4-5 \times 10^5$  cells per well) and 50  $\mu$ L of the  
 25 antigen solution were added. To the control well, 50  $\mu$ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4  $\mu$ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790)  
 30 or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap  
 35 by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

*Non-human Primate anti-RT ELISA* - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

*Results - Rodent Studies* - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>7</sup> vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.



Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

| Group | Vaccine               | Dose               | No. of Doses | Anti-RT IgG Titers <sup>a</sup>              |                 |                | SFC/10 <sup>6</sup> cells <sup>b</sup> |                   |                      |
|-------|-----------------------|--------------------|--------------|----------------------------------------------|-----------------|----------------|----------------------------------------|-------------------|----------------------|
|       |                       |                    |              | GMT                                          | +SE             | -SE            | Medium                                 | CD4+ peptide pool | CD8+ peptide pool    |
| 1     | MRKAd5hCMVFLpol (E3+) | 10 <sup>7</sup> vp | 2<br>1       | 310419<br>819                                | 301785<br>372   | 153020<br>265  | 1(1)<br>1(1)                           | 75(4)<br>72(9)    | 2313(87)<br>533(41)  |
| 2     | MRKAd5hCMVFLpol (E3+) | 10 <sup>9</sup> vp | 2<br>1       | 1838400 <sup>b</sup><br>713155               | 0<br>528520     | 0<br>303566    | 2(2)<br>1(1)                           | 114(9)<br>48(7)   | 2083(182)<br>733(89) |
| 3     | MRKAd5hCMVFLpol (E3-) | 10 <sup>7</sup> vp | 2<br>1       | 310419<br>6400                               | 386218<br>14013 | 172097<br>4393 | 0(0)<br>10(8)                          | 223(7)<br>141(21) | 2807(27)<br>409(28)  |
| 4     | MRKAd5hCMVFLpol (E3-) | 10 <sup>9</sup> vp | 2<br>1       | 1838400 <sup>b</sup><br>1241675 <sup>b</sup> | 0<br>396725     | 0<br>300861    | 1(1)<br>0(0)                           | 160(13)<br>39(13) | 2385(11)<br>833(83)  |
| 5     | Naïve                 | none               | none         | 57                                           | 9               | 7              | 9(2)                                   | 11(4)             | 10(1)                |

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>Near or at the upper limit of the serial dilution; hence, could be greater than this value<sup>c</sup>No. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

| Group | Vaccine               | Dose               | No. of Doses | Anti-nef IgG Titers <sup>a</sup> |           |          | SFC/10 <sup>6</sup> cells <sup>b</sup> |                    |               |
|-------|-----------------------|--------------------|--------------|----------------------------------|-----------|----------|----------------------------------------|--------------------|---------------|
|       |                       |                    |              | GMT                              | +SE       | -SE      | Medium                                 | aa51-70 CD8+       | aa81-100 CD4+ |
| 1     | MRKAd5hCMVFLnef (E3+) | 10 <sup>7</sup> vp | 2<br>1       | 174<br>132                       | 70<br>42  | 50<br>32 | 1(1)<br>0(0)                           | 23(1)<br>0(0)      | 1(1)<br>0(0)  |
| 2     | MRKAd5hCMVFLnef (E3+) | 10 <sup>9</sup> vp | 2<br>1       | 174<br>132                       | 70<br>42  | 50<br>32 | 0(0)<br>1(1)                           | 61(7)<br>62(7)     | 4(2)<br>3(1)  |
| 3     | MRKAd5mCMVFLnef (E3+) | 10 <sup>7</sup> vp | 2<br>1       | 132<br>115                       | 42<br>46  | 32<br>33 | 3(1)<br>3(2)                           | 15(5)<br>3(2)      | 5(2)<br>4(2)  |
| 4     | MRKAd5mCMVFLnef (E3+) | 10 <sup>9</sup> vp | 2<br>1       | 132<br>132                       | 42<br>42  | 32<br>32 | 4(2)<br>2(1)                           | 83(13)<br>28(2)    | 5(1)<br>4(0)  |
| 5     | MRKAd5mCMVtpanef(E3+) | 10 <sup>7</sup> vp | 2<br>1       | 132<br>100                       | 42<br>0   | 32<br>0  | 3(2)<br>3(1)                           | 14(2)<br>13(4)     | 5(1)<br>10(3) |
| 6     | MRKAd5mCMVtpanef(E3+) | 10 <sup>9</sup> vp | 2<br>1       | 230<br>115                       | 170<br>46 | 98<br>33 | 3(2)<br>7(1)                           | 145(29)<br>151(14) | 4(0)<br>10(0) |
| 7     | Naïve                 | none               | none         | 152                              | 78        | 62       | 21(2)                                  | 18(6)              | 28(3)         |

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>No. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

*Monkey Studies* - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of  $10^9$  vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

| Vaccine (T=0,4 wks)                   | Monkey # | Prebleed |       |       | T=4  |       |       | T=7  |       |       | T=18 |       |       |
|---------------------------------------|----------|----------|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|
|                                       |          | Mock     | Pol L | Pol R | Mock | Pol L | Pol R | Mock | Pol L | Pol R | Mock | Pol L | Pol R |
| MRKAd5hCMV-IAPol(E3+)<br>$10^{11}$ vp | 99C100   | 1        | 0     | 0     | 1    | 38    | 31    | 0    | 52    | 146   | 0    | 49    | 715   |
|                                       | 99C215   | 1        | 2     | 2     | 10   | 88    | 249   | 1    | 109   | 305   | 22   | 88    | 250   |
|                                       | 99D201   | 5        | 5     | 4     | 6    | 149   | 85    | 0    | 40    | 35    | 0    | 35    | 18    |
| MRKAd5hCMV-IAPol(E3+)<br>$10^9$ vp    | 99D212   | 0        | 2     | 0     | 4    | 331   | 114   | 0    | 58    | 14    | 0    | 6     | 6     |
|                                       | 99D180   | 0        | 4     | 2     | 0    | 19    | 182   | 4    | 38    | 156   | 5    | 38    | 108   |
|                                       | 99C201   | 8        | 5     | 21    | 6    | 62    | 62    | 0    | 18    | 32    | 1    | 14    | 65    |
| MRKAd5hCMV-IAPol(E3-)<br>$10^{11}$ vp | 99D239   | 5        | 2     | 2     | 20   | 82    | 172   | 1    | 68    | 114   | 9    | 21    | 40    |
|                                       | 99C186   | 4        | 12    | 6     | 5    | 120   | 421   | 2    | 271   | 489   | 16   | 875   | 530   |
|                                       | 99C084   | 1        | 8     | 9     | 8    | 84    | 484   | 0    | 14    | 238   | 1    | 24    | 264   |
| MRKAd5hCMV-IAPol(E3-)<br>$10^9$ vp    | CC7C     | 10       | 10    | 8     | 12   | 724   | 745   | 4    | 322   | 376   | 4    | 188   | 176   |
|                                       | CD1G     | 2        | 0     | 1     | 5    | 474   | 468   | 0    | 232   | 212   | 0    | 101   | 121   |
|                                       | CD11     | 6        | 6     | 12    | 10   | 98    | 110   | 5    | 60    | 80    | 8    | 25    | 34    |
| Naïve                                 | 083Q     | nd       | nd    | nd    | nd   | nd    | nd    | 4    | 2     | 2     | 2    | 1     | 2     |

nd, not determined  
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

| RT ANTIBODY ASSAY TITERS IN mMU/mL  |     |      |      |      |
|-------------------------------------|-----|------|------|------|
| Vaccine/Monkey T ag                 | T=4 | T=7  | T=12 | T=16 |
| MRKAd5hCMV-IAPol(E3+), $10^{11}$ vp |     |      |      |      |
| 99C100                              | 61  | 1999 | 5928 | 4768 |
| 99C215                              | 81  | 1541 | 2356 | 2767 |
| 99D201                              | 53  | 336  | 539  | 387  |
| MRKAd5hCMV-IAPol(E3+), $10^9$ vp    |     |      |      |      |
| 99D212                              | 10  | 40   | 49   | 68   |
| 99D180                              | <10 | 36   | 79   | 93   |
| 99C201                              | <10 | 37   | 71   | 76   |
| MRKAd5hCMV-IAPol(E3-), $10^{11}$ vp |     |      |      |      |
| 99D239                              | 44  | 460  | 1234 | 1015 |
| 99C186                              | 21  | 233  | 480  | 345  |
| 99C084                              | 235 | 2637 | 2858 | 1626 |
| MRKAd5hCMV-IAPol(E3-), $10^9$ vp    |     |      |      |      |
| CC7C                                | 32  | 175  | 306  | 235  |
| CD1G                                | 20  | 140  | 273  | 419  |
| CD11                                | 15  | 112  | 149  | 237  |

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

| Vaccine (T=0,4 wks)                                   | Monk # | Pre  |     | T=4  |      | T=7  |      | T=16 |      |
|-------------------------------------------------------|--------|------|-----|------|------|------|------|------|------|
|                                                       |        | Mock | Nef | Mock | Nef  | Mock | Nef  | Mock | Nef  |
| MRKAd5hCMV-nef(G2A,LLAA) (E3+)<br>10 <sup>11</sup> vp | CD2D   | 0    | 4   | 31   | 440  | 4    | 368  | 1    | 251  |
|                                                       | CC7B   | 0    | 0   | 2    | 521  | 0    | 178  | 1    | 1522 |
|                                                       | CC61   | 2    | 9   | 31   | 112  | 0    | 108  | 11   | 100  |
| MRKAd5hCMV-nef(G2A,LLAA) (E3+)<br>10 <sup>9</sup> vp  | CC2K   | 9    | 9   | 6    | 52   | 0    | 35   | 0    | 16   |
|                                                       | CD15   | 5    | 4   | 30   | 998  | 2    | 588  | 0    | 434  |
|                                                       | CD16   | 6    | 1   | 8    | 1148 | 0    | 369  | 1    | 212  |
| MRKAd5mCMV-nef(G2A,LLAA) (E3+)<br>10 <sup>11</sup> vp | 99D191 | 1    | 5   | 4    | 614  | 0    | 298  | 2    | 419  |
|                                                       | 99D144 | 4    | 6   | 5    | 434  | 0    | 1100 | 2    | 932  |
|                                                       | 99C193 | 1    | 2   | 1    | 58   | 1    | 22   | 0    | 64   |
| MRKAd5mCMV-nef(G2A,LLAA) (E3+)<br>10 <sup>9</sup> vp  | 99D224 | 1    | 11  | 14   | 231  | 1    | 125  | 0    | 70   |
|                                                       | 99D250 | 8    | 9   | 4    | 108  | 0    | 54   | 0    | 5    |
|                                                       | 99C120 | 1    | 6   | 20   | 299  | 0    | 92   | 0    | 79   |
| Naive                                                 | 083Q   | nd   | nd  | 18   | 22   | 4    | 5    | 2    | 1    |

#### EXAMPLE 25

- 15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects
- PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-
- 20 b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were
- 25 about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15  
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

| subject | bleed date | gag epitope #<br>(from mapping) | mock | gag H-b | gagH-c | nef-b | nef-c |
|---------|------------|---------------------------------|------|---------|--------|-------|-------|
| #100    | 19-Jul-99  | 12                              | 10   | 3950    | 1385   | 1295  | 1300  |
| #101    | 25-Jul-99  | 3                               | 15   | 3885    | 1280   | na    | 1020  |
| #102    | 25-Jul-99  | 4                               | 15   | 1740    | 850    | 1255  | 1785  |
| #104    | 7-Jun-99   | 2                               | 5    | 1355    | 1185   | na    | 1060  |
| #107    | 11-Oct-99  | 2                               | 25   | 3305    | 2795   | 670   | 870   |
| #405    | 11-Jul-99  | 2                               | 15   | 4575    | 3180   | 1700  | 1500  |
| #501    | 19-Jul-99  | 2                               | 15   | 1100    | 570    | 3365  | 3460  |
| #505    | 18-Jul-99  | 5                               | 10   | 2145    | 1725   | 1235  | na    |
| #506    | 28-Feb-99  | 2                               | 25   | 150     | 45     | 400   | 610   |
| #701    | 28-Mar-99  | 5                               | 30   | 7620    | 4775   | 3320  | 2780  |
| #709    | 17-May-99  | 3                               | 15   | 2785    | 1945   | 1090  | 1630  |
| #710    | 24-May-99  | 4                               | 5    | 1055    | 1080   | 2210  | 2140  |
|         |            |                                 |      |         |        |       |       |

10

#### EXAMPLE 26

#### Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

*Expansion of nef and pol Adenovectors* - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

| Adenovector        | AEX Titer<br>(10 <sup>10</sup> vp/ml culture) | AEX Titer<br>(10 <sup>4</sup> vp/cell) | Amplification<br>Ratio |
|--------------------|-----------------------------------------------|----------------------------------------|------------------------|
| hCMV-FL-nef [E3+]  | 1.1                                           | 0.9                                    | 30                     |
| mCMV-FL-nef [E3+]  | 2.2                                           | 2.1                                    | 75                     |
| hCMV-tpa-nef [E3+] | 0.07                                          | 0.1                                    | 5                      |
| mCMV-tpa-nef [E3+] | 1.3                                           | 0.9                                    | 35                     |
| hCMV-FL-pol [E3+]  | 2.7                                           | 2.1                                    | 75                     |
| hCMV-FL-pol [E3-]  | 1.9                                           | 1.3                                    | 45                     |

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

|                   |      | Xviable (10 <sup>6</sup> cells/ml), Viability (%) |           | Cell Passage Number | ABX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture | Titer 10 <sup>4</sup> vp/cell | Amplification Ratio | Triton Lysis Titer 10 <sup>10</sup> vp/ml culture |
|-------------------|------|---------------------------------------------------|-----------|---------------------|------------------------------------------------------------|-------------------------------|---------------------|---------------------------------------------------|
|                   |      | Infection                                         | Harvest   |                     |                                                            |                               |                     |                                                   |
| hCMV-FL-nef [E3+] | pool | 1.22, 85%                                         |           | 62                  | 0.8                                                        | 0.7                           | 25                  | 1.6                                               |
|                   | 1    |                                                   | 0.99, 62% |                     |                                                            |                               |                     |                                                   |
|                   | 2    |                                                   | 1.10, 72% |                     |                                                            |                               |                     |                                                   |
| hCMV-FL-pol [E3+] | pool | 1.42, 89%                                         |           | 62                  | 4.5                                                        | 3.2                           | 115                 | 7.0                                               |
|                   | 1    |                                                   | 1.22, 70% |                     |                                                            |                               |                     |                                                   |
|                   | 2    |                                                   | 1.42, 74% |                     |                                                            |                               |                     |                                                   |

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

|                   |      | Xviable (10 <sup>6</sup> cells/ml), Viability (%) |           | Cell Passage Number | ABX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture | Titer 10 <sup>4</sup> vp/cell | Amplification Ratio | Triton Lysis Titer 10 <sup>10</sup> vp/ml culture |
|-------------------|------|---------------------------------------------------|-----------|---------------------|------------------------------------------------------------|-------------------------------|---------------------|---------------------------------------------------|
|                   |      | Infection                                         | Harvest   |                     |                                                            |                               |                     |                                                   |
| hCMV-FL-nef [E3+] | Pool | 1.33, 90%                                         |           | 66                  | 1.0                                                        | 0.8                           | 29                  | 2.1                                               |
|                   | 1    |                                                   | 0.96, 70% |                     |                                                            |                               |                     |                                                   |
|                   | 2    |                                                   | 1.18, 73% |                     |                                                            |                               |                     |                                                   |
| hCMV-FL-pol [E3+] | Pool | 0.90*, 90%                                        |           | 56                  | 4.2                                                        | 4.7                           | 168                 | 6.5                                               |
|                   | 1    |                                                   | 1.18, 88% |                     |                                                            |                               |                     |                                                   |
|                   | 2    |                                                   | 1.04, 80% |                     |                                                            |                               |                     |                                                   |

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
- 20 MRKAd5gag. PER.C6<sup>®</sup> cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were
- 25 harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

- 5 *Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the
- 10 four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6<sup>®</sup> cells- experiments are underway at V&CB to measure nef expression levels.

15

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

|                         |      | Xv (10 <sup>6</sup> cells/ml), Viability (%) |           | Cell Passage Number | AEX Titer 10 <sup>10</sup> vp/ml culture | Titer 10 <sup>6</sup> vp/cell | Amplification Ratio | Triton Lysis Titer 10 <sup>10</sup> vp/ml culture |
|-------------------------|------|----------------------------------------------|-----------|---------------------|------------------------------------------|-------------------------------|---------------------|---------------------------------------------------|
|                         |      | Infection                                    | Harvest   |                     |                                          |                               |                     |                                                   |
| hCMV-FL-nef (MRKAd5nef) | Pool | 1.11, 91%                                    |           | 60                  | 1.5                                      | 1.4                           | 50                  | 2.8                                               |
|                         | 1    |                                              | 1.23, 75% |                     |                                          |                               |                     |                                                   |
|                         | 2    |                                              | 1.34, 74% |                     |                                          |                               |                     |                                                   |
| mCMV-FL-nef             | Pool | 1.11, 91%                                    |           | 60                  | 2.3                                      | 2.1                           | 75                  | 4.6                                               |
|                         | 1    |                                              | 1.49, 84% |                     |                                          |                               |                     |                                                   |
|                         | 2    |                                              | 1.18, 77% |                     |                                          |                               |                     |                                                   |

20

#### EXAMPLE 27

##### Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

- Materials and Methods* - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate,
- 25 no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6<sup>®</sup> cells at a concentration of 0.2x10<sup>6</sup> cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10<sup>6</sup> cells/ml. The cells were infected with uncloned
- 30 MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

|             |         |
|-------------|---------|
| Temperature | 36.5 °C |
| DO          | 30%     |
| PH          | 7.30    |
| Agitation   | 150 rpm |
| Sparging    | None    |

Table 21: Virus source used for experiments.

| Run | Batch ID    | Cloned/Unclosed MRKAd5nef | MOI (vp/cells) |
|-----|-------------|---------------------------|----------------|
| #1  | B20010115-1 | Unclosed                  | 280            |
|     | B20010115-2 | Unclosed                  | 280            |
| #2  | B20010202-1 | Cloned                    | 280            |
|     | B20010202-2 | Cloned                    | 280            |

*Results* - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

| Run | Batch ID    | Cloned/Unclosed MRKAd5nef | Virus Concentration @ 48hpi (1x10 <sup>13</sup> vp/L) |                  |       |               |
|-----|-------------|---------------------------|-------------------------------------------------------|------------------|-------|---------------|
|     |             |                           | Supernatant                                           | Clarified Lysate | Total | Triton Lysate |
| #1  | B20010115-1 | Unclosed                  | 0.72                                                  | 3.26             | 3.98  | 5.76          |
|     | B20010115-2 | Unclosed                  | 0.38                                                  | 1.67             | 2.05  | 2.46          |
| #2  | B20010202-1 | Cloned                    | 0.80                                                  | 6.00             | 6.80  | 8.88          |
|     | B20010202-2 | Cloned                    | 0.50                                                  | 6.00             | 6.50  | 8.47          |

Table 23: Virus Titers as measured by the QPA assay

| Run | Batch ID    | Cloned/Unclosed MRKAd5nef | Virus Concentration @ 48hpi (1x10 <sup>11</sup> IU/L) |             |                  |       |               |
|-----|-------------|---------------------------|-------------------------------------------------------|-------------|------------------|-------|---------------|
|     |             |                           | Whole Broth                                           | Supernatant | Clarified Lysate | Total | Triton Lysate |
| #1  | B20010115-1 | Unclosed                  | 0.13                                                  | 1.12        | 1.76             | 2.88  | 11.28         |
|     | B20010115-2 | Unclosed                  | 0.14                                                  | 0.73        | 1.54             | 2.27  | 5.86          |
| #2  | B20010202-1 | Cloned                    | 0.14                                                  | 0.97        | 1.62             | 2.69  | 11.89         |
|     | B20010202-2 | Cloned                    | 0.14                                                  | 1.17        | 1.70             | 2.97  | 12.47         |

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

#### EXAMPLE 28

##### MRKAd5HIV-1gag Boosting of DNA-Primed Animals

5 Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of  
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of  $10^7$  viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note:  $10^7$  is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50  
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced,  $CD4^+$ -biased or  $CD8^+$ -biased, and (b) boosting with the MRKAd5gag  
30 construct produced in all cases a strongly  $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific  $CD8^+$  T cells.



Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

| Grp | Priming<br>T=0, 4, 8 wks<br>DNA/5 mgs<br>PBS<br>(D101) | Boost<br>T=28 wks<br>MRKAd5gag(E3+)<br>10 <sup>7</sup> vp | Monkey | T=0              |       |       | T=4    |       |       | T=6    |       |       | T=10   |       |       | T=17   |       |       | T=24   |       |       | T=28   |       |       | T=30   |       |       |
|-----|--------------------------------------------------------|-----------------------------------------------------------|--------|------------------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|
|     |                                                        |                                                           |        | Medium           | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H | Medium | gag H | gag H |
| 1   |                                                        |                                                           |        | NA               | 0     | 0     | 3      | 35    | 15    | 71     | 4     | 224   | 8      | 115   | 8     | 05     | 19    | 956   | 0      | 316   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB5H             | 0     | 15    | 0      | 15    | 0     | 48     | 0     | 58    | 0      | 76    | 0     | 35     | 3     | 1705  | 1      | 755   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB5K             | 5     | 11    | 0      | 36    | 3     | 51     | 3     | 48    | 2      | 89    | 8     | 65     | 10    | 989   | 0      | 395   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | AK8B             |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
| 2   |                                                        |                                                           |        | 0                | 4     | 4     | 1      | 60    | 0     | 111    | 5     | 270   | 4      | 280   | 8     | 232    | 3     | 959   | 19     | 1345  |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CC1C             | 0     | 4     | 1      | 101   | 0     | 294    | 0     | 781   | 5      | 452   | 0     | 321    | 0     | 1915  | 1      | 1099  |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CC1K             | 9     | 8     | 1      | 10    | 4     | 71     | 4     | 164   | 8      | 104   | 5     | 85     | 11    | 836   | 8      | 241   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | AW3P             | NA    | NA    | 0      | 31    | 0     | 268    | 0     | 530   | 19     | 374   | 8     | 251    | 8     | 1549  | 20     | 1734  |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB5F             | 9     | 12    | 4      | 38    | 1     | 119    | 0     | 439   | 0      | 425   | 0     | 316    | 4     | 1229  | 5      | 1354  |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | AK8B             |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
| 3   |                                                        |                                                           |        | 10               | 4     | 4     | 1      | 59    | 5     | 284    | 19    | 425   | 6      | 105   | 9     | 205    | 19    | 565   | 8      | 404   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | AW2D             | 1     | 135   | 0      | 121   | 1     | 135    | 1     | 270   | 5      | 130   | 1     | 105    | 14    | 1384  | 10     | 978   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CA4R             | 8     | 6     | 0      | 6     | 3     | 119    | 0     | 274   | 6      | 282   | 1     | 208    | 0     | 638   | 1      | 828   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB5B             | 4     | 3     | 0      | 26    | 1     | 91     | 0     | 139   | 0      | 164   | 1     | 62     | 5     | 543   | 1      | 349   |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB5W             | 1     | 0     | 0      | 136   | 0     | 318    | 1     | 609   | 5      | 628   | 1     | 759    | 0     | 2278  | 4      | 1831  |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | CB7D             |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
| 4   |                                                        |                                                           |        | 3                | 0     | 0     | 0      | 0     | 1     | 0      | 0     | 0     | 0      | 1     | 1     | 2      | 3     | 0     | 0      | 0     |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | 98D201           |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | None             |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | None             |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |
|     |                                                        |                                                           |        | NA not available |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |        |       |       |

NA, not available

## EXAMPLE 29

## Construction of gagpol fusion for MRKAd5gagpol fusion constructs

5 The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the LA pol gene (consisting of RT, RNaseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the LApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-LApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR  
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-LApol fusion gene.

## EXAMPLE 30

## 20 Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of  
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein  
30 sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels  
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

**Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

| Grp # | Vaccine<br>T=0, 4 wks                                     | Monk # | T=5 wks |       |         |         |      |
|-------|-----------------------------------------------------------|--------|---------|-------|---------|---------|------|
|       |                                                           |        | Mock    | Gag H | Pol - 1 | Pol - 2 | Nef  |
| 1     | MRKAd5 gag<br>10 <sup>10</sup> vp                         | CB9V   | 0       | 15    | -       | -       | -    |
|       |                                                           | CD19   | 0       | 374   | -       | -       | -    |
|       |                                                           | 109H   | 1       | 843   | -       | -       | -    |
| 2     | MRKAd5 gag<br>10 <sup>8</sup> vp                          | 99D130 | 1       | 948   | -       | -       | -    |
|       |                                                           | W277   | 16      | 324   | -       | -       | -    |
|       |                                                           | 143H   | 4       | 595   | -       | -       | -    |
| 3     | MRKAd5 pol<br>10 <sup>10</sup> vp                         | CC1X   | 4       | -     | 46      | 256     | -    |
|       |                                                           | AW3W   | 3       | -     | 463     | 550     | -    |
|       |                                                           | AV43   | 6       | -     | 95      | 1333    | -    |
| 4     | MRKAd5 pol<br>10 <sup>8</sup> vp                          | AW38   | 1       | -     | 19      | 30      | -    |
|       |                                                           | CC8K   | 0       | -     | 50      | 995     | -    |
|       |                                                           | CC21   | 1       | -     | 33      | 436     | -    |
| 5     | MRKAd5 nef<br>10 <sup>10</sup> vp                         | 076Q   | 9       | -     | -       | -       | 1204 |
|       |                                                           | 091Q   | 4       | -     | -       | -       | 85   |
|       |                                                           | 083Q   | 0       | -     | -       | -       | 176  |
| 6     | MRKAd5 nef<br>10 <sup>8</sup> vp                          | 00C029 | 1       | -     | -       | -       | 114  |
|       |                                                           | 98D022 | 6       | -     | -       | -       | 170  |
|       |                                                           | 98D160 | 3       | -     | -       | -       | 198  |
| 7     | MRKAd5gag+MRKAd5pol+MRKAd5nef<br>10 <sup>10</sup> vp each | 99D251 | 3       | 206   | 15      | 193     | 120  |
|       |                                                           | 05H    | 3       | 135   | 21      | 9       | 638  |
|       |                                                           | 00C016 | 3       | 26    | 4       | 51      | 23   |
| 8     | MRKAd5gag+MRKAd5pol+MRKAd5nef<br>10 <sup>8</sup> vp each  | 99D215 | 1       | 171   | 18      | 193     | 240  |
|       |                                                           | 81H    | 5       | 73    | 6       | 14      | 243  |
|       |                                                           | 12H    | 8       | 1140  | 115     | 811     | 719  |
| 9     | MRKAd5gagpol +MRKAd5 nef<br>10 <sup>10</sup> vp each      | 99D211 | 0       | 83    | 56      | 838     | 725  |
|       |                                                           | 22H    | 4       | 385   | 119     | 1194    | 1915 |
|       |                                                           | 61H    | 4       | 343   | 11      | 765     | 853  |
| 10    | MRKAd5gagpol +MRKAd5 nef<br>10 <sup>8</sup> vp each       | 34H    | 3       | 78    | 19      | 5       | 75   |
|       |                                                           | 48H    | 1       | 65    | 105     | 46      | 43   |
|       |                                                           | 70H    | 5       | 158   | 15      | 220     | 191  |

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10<sup>6</sup> PBMC.

## WHAT IS CLAIMED IS

1. A recombinant adenoviral vaccine vector at least partially deleted in  
5 E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from  
about base pair 1 to between from about base pair 400 to about  
base pair 458 of a wildtype adenovirus genome; and
  - b) a gene encoding an HIV protein or immunologically relevant  
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region  
corresponding to from about base pair 1 to about base pair 450 of a wildtype  
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides  
15 corresponding to between from about base pair 3511 to about 3524 to about base pair  
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs  
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs  
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially  
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region  
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a  
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene  
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).
18. A cell comprising the adenoviral vector of claim 1.
19. Recombinant, replication-defective adenovirus particles harvested  
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.
20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
21. An HIV vaccine composition of claim 20 which comprises a  
10 physiologically acceptable carrier.
22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,  
15 replication-defective adenovirus.
23. A method according to claim 22 wherein the cell is a PER.C6<sup>®</sup> cell.
24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
20 claim 21.
25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
  - i) SEQ ID NO: 29;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5           34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10           36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell  
15   line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20           41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.



42. A method according to claim 41 wherein the cell is a PER.C6<sup>®</sup> cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

20 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus  
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of  
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6<sup>®</sup> cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with  
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5           67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10           69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

20           70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5           73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10           75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15           77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

20           79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

80. A method according to claim 79 wherein the cell is a PER.C6<sup>®</sup> cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- 5
- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- 10
- f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- 15
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- 20
- k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;

and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with  
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with  
claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with  
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to a single promoter; and the encoding nucleic acid sequences  
operatively linked by an internal ribosome entry sequence ("IRES").



Original Adenovector Construct:

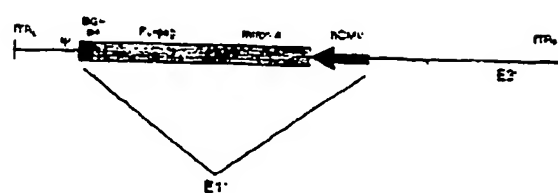


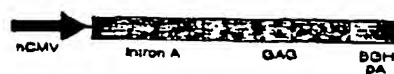
Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

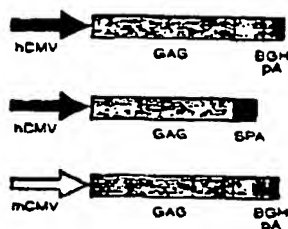
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agggctgtggaagtgtggcaaggaggccaccagatgaaggactgcaatgagaggcaggccaacttctg  
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gat (SEQ ID NO: 29)

Figure 2

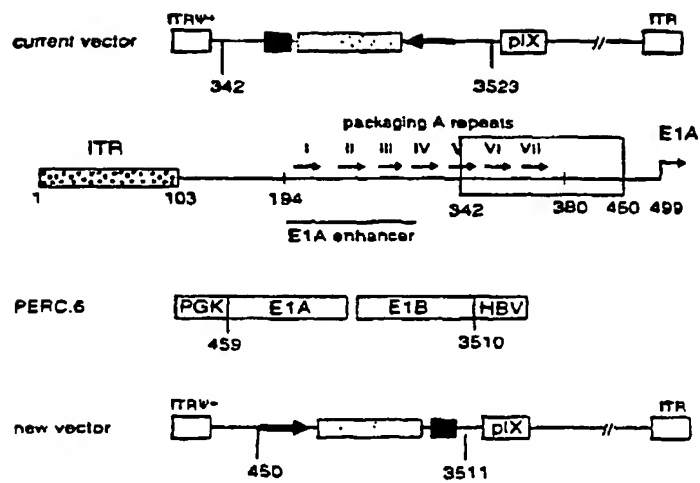
Old Transgene:



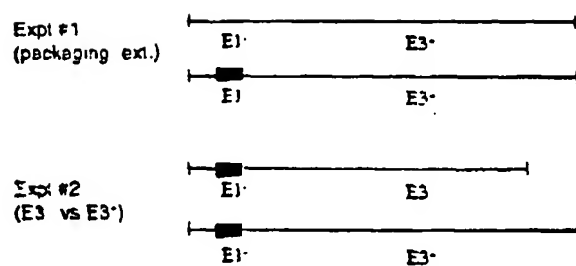
New Transgenes:



**Figure 3:** Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.



**Figure 4:** Modifications made to the current adenovector backbone in the generation of the new vector.



**Figure 5:** Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

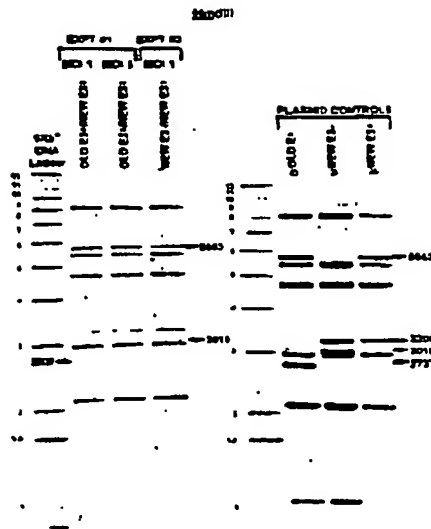
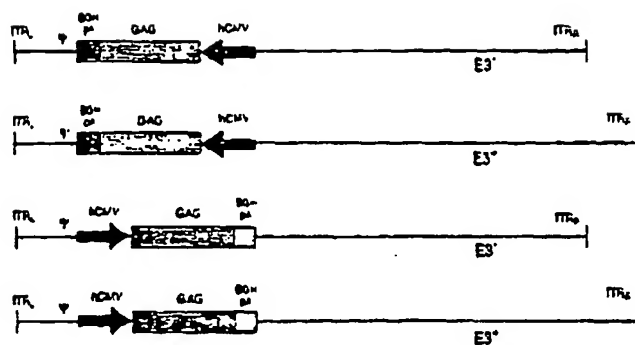
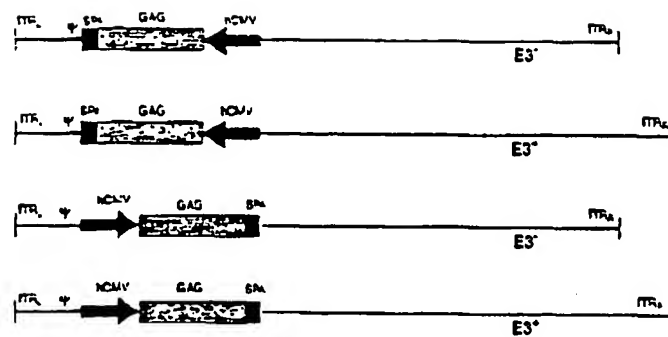


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

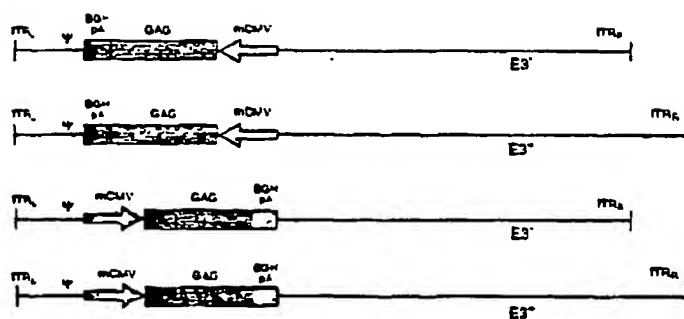


**Figure 7A:** hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.



**Figure 7B:** hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3<sup>-</sup> and E3<sup>+</sup> backbones were constructed.





**Figure 7C:** mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

## Plasmid mixing expt: (orientation)

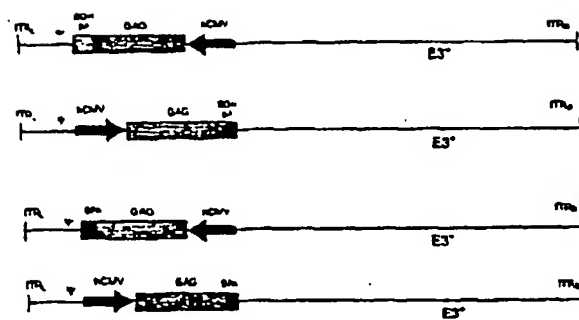


Figure 8A: Effect of transgene orientation

## Plasmid Mixing expt: (poly A signal)

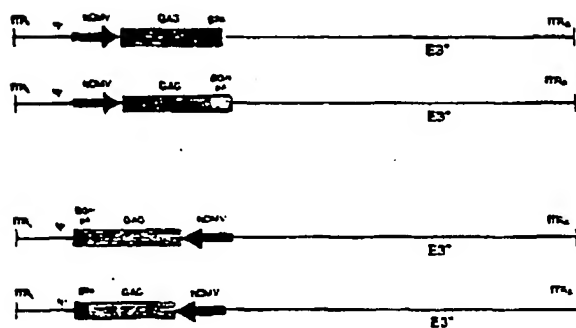


Figure 8B: Effect of polyadenylation signal

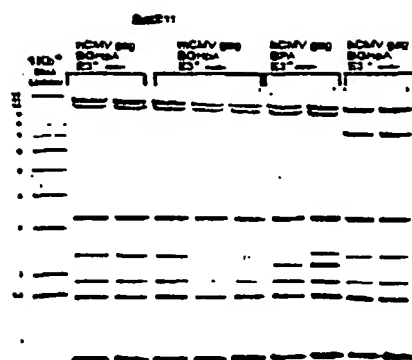
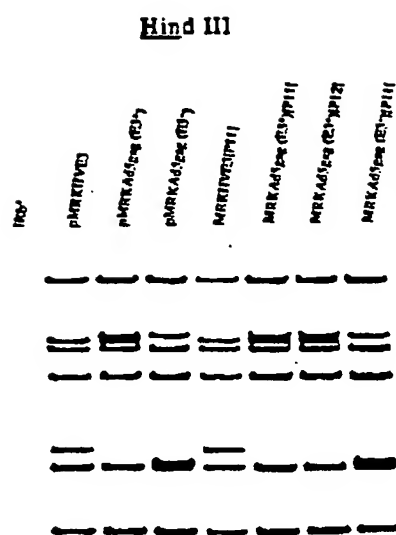


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.



**Figure 10:** Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

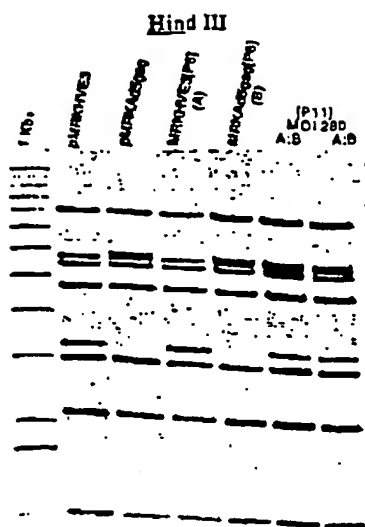
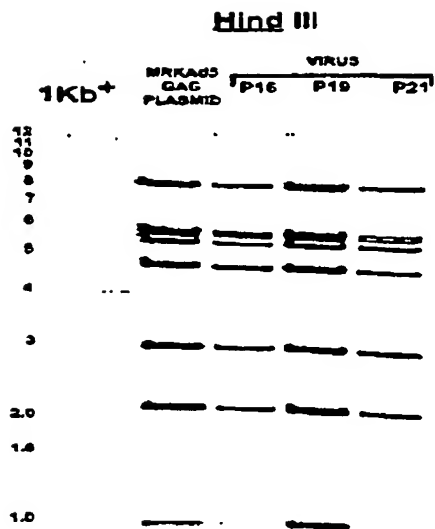
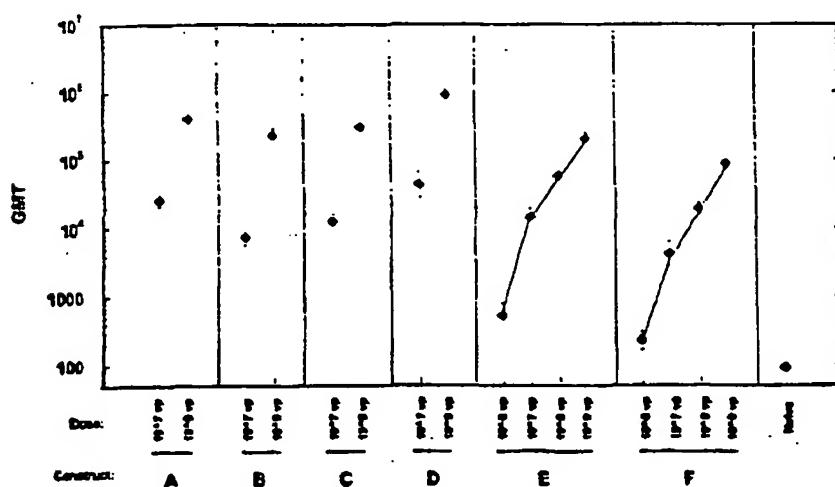


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).



**Figure 12:** Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13  
 Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3' hCMV-FLgag-bGHpA; (C) MRKAd5 E3' hCMV-FLgag-SPA; (D) MRKAd5 E3' mCMV-FLgag-bGHpA; (E) research lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.





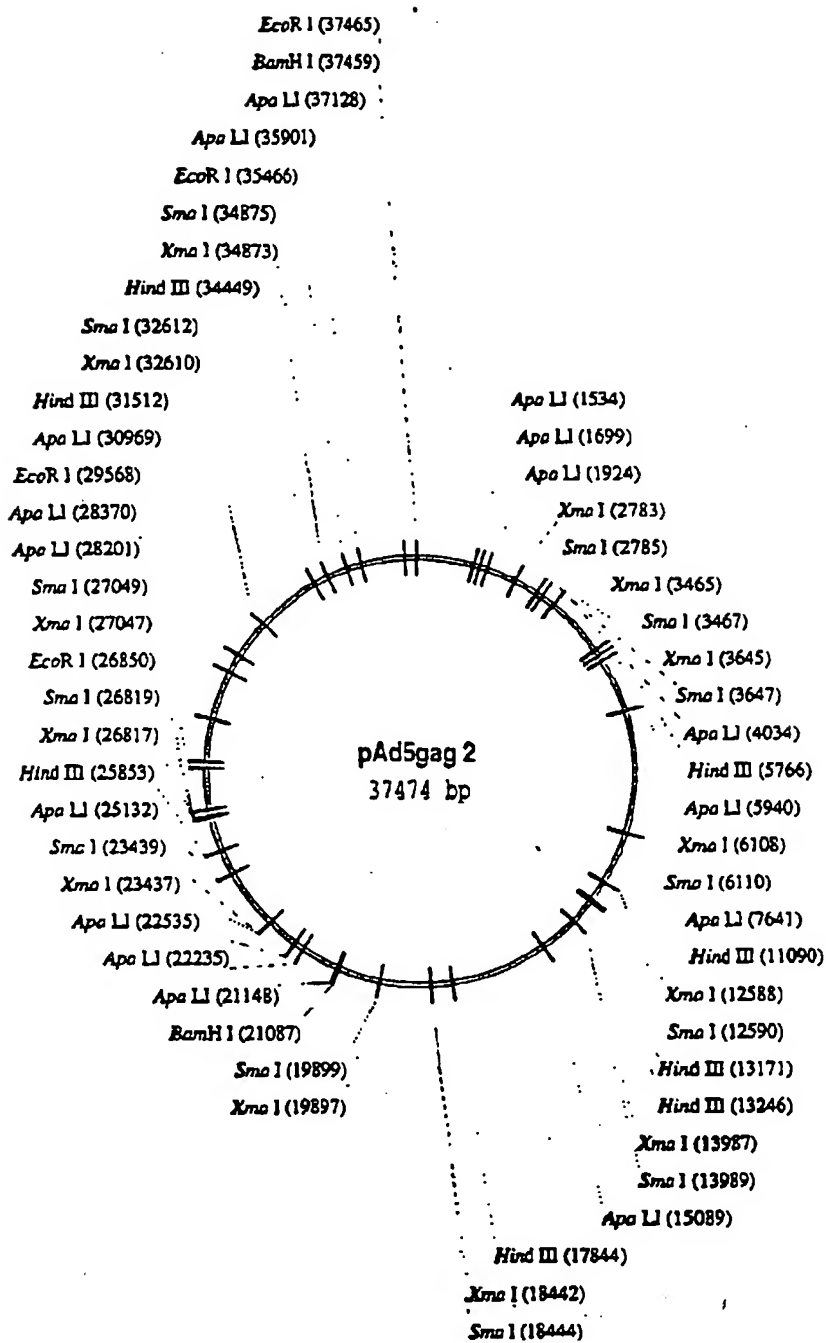


Figure 14



## pNRKau1000 MER62

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 GTGGTCCCGT AGAGCGCGCG CTGGTACTTA CCGAGGAGCT TTATAGTACT CTCTCTCCCG AGAGCGCGAC TCCACTATCG GTACAGAGCA GTTACAGAGC  
 1801 AGCTTCCGAC CCGCCAGGAC CTGAGATATA TTCTGATAC ATCTGATGCT CATATGCTG GTATGCTGCT GTATGCTGCT ACCATCAATG AATAGGCTT  
 TCCAGCGTGG AGCTTCCGCG GACTTCTCTT ACTATCTCTG TCTATCTCTG GTATGCTGCT GTATGCTGCT GTATGCTGCT GTATGCTGCT GTATGCTGCT  
 1901 TCACTGCGGAC AGCTTCCGCG GACTTCTCTT ACTATCTCTG TCTATCTCTG GTATGCTGCT GTATGCTGCT GTATGCTGCT GTATGCTGCT GTATGCTGCT  
 ACTGAGCGCG TCCAGCGTGG GACAGCTCTG ACCGCTCTCT ACTGAGCGCG TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC  
 2001 CAGGAGCAGA TTGGCTCGAT GAGCAGGAGC CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG  
 GTCTCTCTCT TACCGAGCTA CTGGTCTCTG GTAGAGAGCG GTAGAGAGCG GTAGAGAGCG GTAGAGAGCG GTAGAGAGCG GTAGAGAGCG GTAGAGAGCG  
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 GTCTCTCTCT TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC TCACTGCGGAC  
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Figure 15B

[illegible]

Figure 15c

## pHMKAd5gag MER602

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 CCAGCGCAAC TCCGACCAAG ACTGACCAAG CTTCCTGATC GCTCAAGCG GATCTGCTAG CCGTCTATC GTAAACTGCT ACCACAGTAT CAGTCTGGGT  
 5001 TCCCGCGCTT GCGCTTTGCG GCGCAGTTGG CCGTTGCTAT ATGCTCTGCA CTGCTGCTAG TCAAGCGCTA GAGCTTTGCG GCTACAGATA  
 AGCGCGCGCA CCGCGAAGCG CCGCTGCAAC GCGAACTGCG TCGCTGCTAT CTCTGCTGCT AGCTCTGCTA ACTTCCGCGT CTTGAGCGCG CCGCTTTTAT  
 5101 CCGATTCCCG GGAATTAGCA TCCGCGCGCG AGGCTGCTCA GATTGCTTCT CATTTGACCA CTGCTGCTAG TCGGCTGCTA AAATCAGGTT  
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 5201 TCCCGCATGC TTGTTGATGC GTTCTTACG TCTGTTTTC ATGATGCTGT GTCCAGTCTC GGTGACGAAA AGGCTGCTCG TGTTGCGCTA TACAAGCTTT  
 AGCGGTACG AAAAATCAG CAAGAGATCG AGACCAAGCG TACTGCTGCA CAGCTGCGAG CCACTGCTTT TCCGACAGCG ACAGGCGCAT ATGCTCTGAA  
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 TCACTGCTG CATCGCGCG AACAGCTGAT CCGCGCGG AGCTGCTGCT CACACTTCTG TGTACAGCG GAGAGCGCTT AGTCTGCTGCT ACTAAGCAGA  
 5501 GTAGGCTGAG GCGCTGCTG CCGCTGCTG TGAAGGCTG CTATAAAGG GATCTGCTG CCGCTGCTG TCACTGCTG CCGCATGCTT GCTGCGAGT  
 CATCCAGTC CCGTGCATG GCGCAGAG ACTTCCCGG GATATTTTC CCGACCGCTG CCGAGCGAG AGTGAGAGAA GCGGTAGCA CAGAGCGCTC  
 5601 GCGAGCTGTT GCGGTGAGTA GTGCTGCTG AAGCGCGCA TCACTGCTG GATAAGATG TCAAGTTTCA AAAAGCGGA GGAATGATA TTCACTGCT  
 CCGTGCAGAA CCGCACTCAT GAGCGAGCT TTTCGCGCTT ACTGAGAGG GATCTGCTG AGTCAAGCTT TTTCGCTGCT CCTTAAGCTAT AAATGAGCTT  
 5701 CCGCGGTAT GCGTTGAGG GTGCGCGCAT CCACTGCTG AGAAGAGCA ATCTTTTGT TGTCAAGCTT GGTGCGAAC GAGCGGTAGA GGTGCTGTA  
 GCGCGCACTA CGGAAGCTC CAGCGCGCTA CAGCGCGCTA TCTTTCTGT TAGAAGAGCA ACAGTTGCAA CCACTGCTG CCGCGCATCT CCGCGAGCT  
 5801 CAGCACTTGG CCGATGAGC GCGAGTTTG GTTTGCTG CCACTGCTG GCTCTGCTG CCGTATGTTT AGCTGAGCTT ATTGCGCGC AAGCGAGCT  
 GTCTGTTGAC CCGTACTCTG CCGTGCAGC CAAAGAGCG GCGTACAGCG CCGCTACAAA TCGATGCTGA TCGAGCTGCTA TAAAGCGCGG TTGCGTGTG  
 5901 CATTCGGGAA AGAGGCTGTT GCGCTGCTG GCGAGCTGTT GCGCTGCTG ACTGCTGCTA ACTGCTGCTG TCGAGGCTA CAGCTGCTG ACTGCTGCT  
 GTAGGCGCTT TCTGCGCA CCGGAGCG CCGTGTGCA GCTGCTGCTT TCGCGCAC ACTTCCACT GTTCCAGTTG CCGACACCGA TCGAGAGCT  
 6001 GTAGCGCTG GTTGTGCGAG CAGAGCTG CCGCTGCTG GCGCTGCTG GATGCTGAGG CCGCTGCTG GCGCTGCTG CCGCGCTG CCGCTGCTG  
 CATCGCGGAG CAGGAGCTC GTCTGCTG CCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG  
 6101 AAGAGCGCG GCGAGCGCG GCGCTGCTG CAGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG  
 TTCTGCGCG CCGCTGCTG CCGCTGCTG CAGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG  
 6201 GCGTGTGAGT GCGGAGCGCA TCGGAGCTG TCGGAGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG  
 CCGCACTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 6301 ATGTAGGCTA CCACTGCTG CCGCTGCTG TCGGAGCTG TCGGAGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG GCGCTGCTG  
 TACATCTG CCGAGAGCT GCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 6401 CCGCTGCTG CCGAGAGCT TCTGCTGAG GATGCTGAT ATAGGCTG AGCTGAGG AGCTGAGG AGCTGAGG AGCTGAGG AGCTGAGG AGCTGAGG  
 GAGGAGGAG GCGCTGCTG AGAGAGCT CAGCGCTG CAGCGCTG CAGCGCTG CAGCGCTG CAGCGCTG CAGCGCTG CAGCGCTG CAGCGCTG

Figure 150

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6501 GCGTCACGCA CCGAGGAGGC GTACAGGTGC CTACAGTTCT TCACTAGTTC GCGCTTCAGC TGCAGTCTTA GCGGCGAGTA GCGGCGAGTA GTCCAGGTTT TCTTCGATCA  
 CCGAGTGGCT GCTTCCTCCG CATCTCTAGC GGTCTGACCA ACTGATGAG CCGCTACTAT CCGGCTGCTT CCGGCTGCTT CCGGCTGCTT CCGGCTGCTT CCGGCTGCTT  
 6601 TGTGATACCT ATCTGCTCCG TTTTCTTCC AGAGCTCTCT GTAGAGTA AATTTCTC GGTCTCTTCA GTACTCTTTC GTACTCTTTC GTACTCTTTC GTACTCTTTC  
 ACAGTATGAA TGGAGAGGCG AAAAAAGG TGTGAGTTC CAAGCTCTCT TGTAGAGGCG CCGAGAGGCT CCGAGAGGCT CCGAGAGGCT CCGAGAGGCT CCGAGAGGCT  
 6701 CCGAGCTGAA GAGCTGAGCA TGTATACCTG GTTACAGGCG TGTAGAGGCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT  
 GGTGCTGCTT CTTGAGCTCT ACATCTCTCT CACTCTCTCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT  
 6801 GAGGTGTGGG TGAAGCGAAA GGTGCTGCTT AGATCTCTCT CACTCTCTCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT AGATCTCTCT TGTAGAGGCT  
 CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT CTTGAGCTCT  
 6901 GGTGCTGCTT TTTGAGCTCT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7001 TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 7101 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7201 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7301 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7401 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7501 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7601 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7701 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7801 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 7901 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT  
 8001 GGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT TGTGCTGCTT  
 GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT GGTGCTGCTT

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|      |             |           |            |            |           |            |            |            |           |           |
|------|-------------|-----------|------------|------------|-----------|------------|------------|------------|-----------|-----------|
| 8101 | ATGCAATCTAA | AGCGGTGAC | GCGTCTGAC  | CTGCTGATAT | ATGCTGCT  | CTGCAACCTG | CGCGGATATG | GGCAGCGGCA | CGTCTGCTG | CGTCTGCTG |
| 8201 | TACGTAGATT  | TTCGCACTG | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8301 | AGGAGCTGCT  | CGTCTGCTG | TACCTCTGCT | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8401 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8501 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8601 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8701 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8801 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 8901 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9001 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9101 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9201 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9301 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9401 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9501 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |
| 9601 | CGCTCTGCT   | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT  | CGCTCTGCT | CGCTCTGCT |

Figure 15F

## pHRKAd5q87 MERG82

|       |            |            |           |           |           |           |           |           |           |
|-------|------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 9701  | ACAAAGCGGT | GGTATGCGCC | GGTGTGATG | GTGTATGTC | AGTGTGAT  | TTAAGGTCT | GGTACCGCG | CTGTGAGAG | TGGTGTATC |
|       | TGTTTCCCA  | CCATACCGG  | GCACACTAC | CATATTCAG | TTAACTGTA | TTATCTGTC | AAATGCGA  | CAACTGCG  | CAACTGTC  |
|       |            |            |           |           |           |           |           |           |           |
| 9801  | TGAGACGGA  | GTAAAGCTC  | GAGTCAGTA | GTATATGCT | GTATATGCT | GTATATGCT | GTATATGCT | GTATATGCT | GTATATGCT |
|       | ACTCTGCGT  | CATTGCGAG  | CTCAGTTAT | GCATGCTTA | GCATGCTTA | GCATGCTTA | GCATGCTTA | GCATGCTTA | GCATGCTTA |
|       |            |            |           |           |           |           |           |           |           |
| 9901  | GGGCTAGCT  | AGGTGCGCG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | CCGCTGCGA  | TCCGACCGC  | CCGCTGCGC | CCGCTGCGC | CCGCTGCGC | CCGCTGCGC | CCGCTGCGC | CCGCTGCGC | CCGCTGCGC |
|       |            |            |           |           |           |           |           |           |           |
| 10001 | GTGCTGAGG  | GGGCTGAGG  | GTGCTGAGG | GTGCTGAGG | GTGCTGAGG | GTGCTGAGG | GTGCTGAGG | GTGCTGAGG | GTGCTGAGG |
|       | CACGACCTC  | GGGCTGCTT  | CAGGCTGCT | GGGCTGCTT | GGGCTGCTT | GGGCTGCTT | GGGCTGCTT | GGGCTGCTT | GGGCTGCTT |
|       |            |            |           |           |           |           |           |           |           |
| 10101 | AACTGTTGAC | GGTCTAGAC  | GTGCAAGAG | AGAGCTGTA | AGAGCTGTA | AGAGCTGTA | AGAGCTGTA | AGAGCTGTA | AGAGCTGTA |
|       | TTAGCAACTG | CGGATCTGG  | CAGCTTTTC | TCTGAGCAT | TCTGAGCAT | TCTGAGCAT | TCTGAGCAT | TCTGAGCAT | TCTGAGCAT |
|       |            |            |           |           |           |           |           |           |           |
| 10201 | GGTGTGAGC  | CCGCTATCC  | GGGCTGCTG | GTATGCTAT | GTATGCTAT | GTATGCTAT | GTATGCTAT | GTATGCTAT | GTATGCTAT |
|       | GGCAAGCTG  | GGGCTATGC  | GGGCTATGC | CAGTATGCT | CAGTATGCT | CAGTATGCT | CAGTATGCT | CAGTATGCT | CAGTATGCT |
|       |            |            |           |           |           |           |           |           |           |
| 10301 | TTGCTGCTC  | TTGCTGCTC  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | AAAGCGAAG  | AAAGTCCCG  | CCGCTGCTG | CCGCTGCTG | CCGCTGCTG | CCGCTGCTG | CCGCTGCTG | CCGCTGCTG | CCGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10401 | GGTCTGCTC  | TGTAGCTG   | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | CGAGCGAGG  | AGATGCTG   | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10501 | CTGCTGCTC  | TGTAGCTG   | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GAGGCGAGT  | AGCTGCTG   | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10601 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10701 | GGGCTGCTC  | TGTAGCTG   | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10801 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 10901 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 11001 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 11101 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |
| 11201 | GGGCTGCTC  | AGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       | GGGCTGCTG  | GGGCTGCTG  | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG | GGGCTGCTG |
|       |            |            |           |           |           |           |           |           |           |

Figure 156





[illegible]

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|       |                                                                                                        |
|-------|--------------------------------------------------------------------------------------------------------|
| 14501 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 14601 | CGATTACT AGACCTGCA CCATTGTAA GCGCTTAA CTTACATTC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC      |
| 14701 | AGATGACAC AGATGACAC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC    |
| 14801 | AGATGACAC AGATGACAC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC GTATATATC    |
| 14901 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15001 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15101 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15201 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15301 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15401 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15501 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15601 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15701 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15801 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 15901 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |
| 16001 | CTTACAGTGA TCTGAGGCT GTTACATTC CCGACATCT GTATGATGAC GTTATATAT GTAGCTTATA AGATGACAC GATCAGGCG GCGTATATC |

Figure 15J

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|       |             |            |            |             |            |            |            |            |            |            |
|-------|-------------|------------|------------|-------------|------------|------------|------------|------------|------------|------------|
| 16101 | CCAGGTCAATC | GGCGCGGAGA | TCATATGCTC | GGCGTAAAGAG | GAAGACAGAG | ATTATTAAGT | CTGAAGCTTA | ANGCGGTCTA | AAAGGAAAAA | GAAGATATAT |
|       | GGTCCAGTAG  | CGCGGCTCT  | AGATACCGAG | GGCGTCTCTC  | CGTATCTCTC | GAATATCTCT | GAATATCTCT | TTTCCGAGT  | TTTCTCTTTT | CTTCTACT   |
|       |             |            |            |             |            |            |            |            |            |            |
| 16201 | GAATGATGAC  | TTGACGACGA | GGTGTAACTG | CTTACGCTTA  | CTTATCTCTC | GAATGATCTA | CAATGATCTA | GTGCGGCTTT | AAAGGCTTTT | TTGCGATCT  |
|       | CTACTACTTT  | AATCTCTCT  | CGACCTCTAC | GAATGATCTA  | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA |
| 16301 | GCACCTACCT  | AGTCTCTTAC | CGCGCTTACG | CGCGCTTACG  | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG |
|       | CGTGTGTGCA  | TCAGAAATTC | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 16401 | CGACGCTCTC  | GGCGCTTACG | CGCGCTTACG | CGCGCTTACG  | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG | CGCGCTTACG |
|       | GGTGTGTGCA  | TCAGAAATTC | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       |             |            |            |             |            |            |            |            |            |            |
| 16501 | CTACGACGAG  | TCGTGCGCGC | GGTGTAACTG | CTTACGCTTA  | CTTATCTCTC | GAATGATCTA | CAATGATCTA | GTGCGGCTTT | AAAGGCTTTT | TTGCGATCT  |
|       | GAATGATCTA  | AGTCTCTTAC | CGACCTCTAC | GAATGATCTA  | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA | GAATGATCTA |
| 16601 | AGCGCTACAG  | ACTGTGATAT | GGTGTAACTG | CTTACGCTTA  | CTTATCTCTC | GAATGATCTA | CAATGATCTA | GTGCGGCTTT | AAAGGCTTTT | TTGCGATCT  |
|       | TCGTGCGCGC  | TCAGAAATTC | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 16701 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 16801 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 16901 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 17001 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 17101 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       |             |            |            |             |            |            |            |            |            |            |
| 17201 | ATATGCGCTT  | CACGTGCGCT | CTCGTCTTCC | GGTGTAACTG  | CTTACGCTTA | CTTATCTCTC | GAATGATCTA | CAATGATCTA | GTGCGGCTTT | AAAGGCTTTT |
|       | TTATACCGGA  | GTGACGCGCG | GAAGCAAGCG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       |             |            |            |             |            |            |            |            |            |            |
| 17301 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
| 17401 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       |             |            |            |             |            |            |            |            |            |            |
| 17501 | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |
|       | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG  | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG | GGCGCTTACG |

Figure 15K

PMRKA157ag MFR682

| Year  | Country | Population (millions) | Urban Population (millions) | Population Density (per sq km) | Life Expectancy (years) | Healthcare Expenditure (% of GDP) | Education Expenditure (% of GDP) | Employment Rate (%) | Unemployment Rate (%) | Gender Inequality Index | Human Development Index | Corruption Perception Index | Trust in Government (%) | Political Freedom Score | Economic Freedom Score | Environmental Quality Index | Peace Index | Global Competitiveness Index | World Economic Forum Global Competitiveness Index |
|-------|---------|-----------------------|-----------------------------|--------------------------------|-------------------------|-----------------------------------|----------------------------------|---------------------|-----------------------|-------------------------|-------------------------|-----------------------------|-------------------------|-------------------------|------------------------|-----------------------------|-------------|------------------------------|---------------------------------------------------|
| 17601 | USA     | 39.2                  | 15.8                        | 35.2                           | 75.4                    | 1.2                               | 2.1                              | 65.3                | 34.7                  | 0.78                    | 0.85                    | 2.5                         | 78.5                    | 7.2                     | 8.1                    | 6.8                         | 8.9         | 7.1                          | 7.5                                               |
| 17701 | USA     | 40.5                  | 16.5                        | 36.0                           | 76.2                    | 1.3                               | 2.2                              | 66.1                | 33.9                  | 0.79                    | 0.86                    | 2.6                         | 79.2                    | 7.3                     | 8.2                    | 6.9                         | 9.0         | 7.2                          | 7.6                                               |
| 17801 | USA     | 41.8                  | 17.2                        | 36.8                           | 77.0                    | 1.4                               | 2.3                              | 66.9                | 33.1                  | 0.80                    | 0.87                    | 2.7                         | 80.0                    | 7.4                     | 8.3                    | 7.0                         | 9.1         | 7.3                          | 7.7                                               |
| 17901 | USA     | 43.1                  | 18.0                        | 37.6                           | 77.8                    | 1.5                               | 2.4                              | 67.7                | 32.3                  | 0.81                    | 0.88                    | 2.8                         | 80.8                    | 7.5                     | 8.4                    | 7.1                         | 9.2         | 7.4                          | 7.8                                               |
| 18001 | USA     | 44.4                  | 18.8                        | 38.4                           | 78.6                    | 1.6                               | 2.5                              | 68.5                | 31.5                  | 0.82                    | 0.89                    | 2.9                         | 81.6                    | 7.6                     | 8.5                    | 7.2                         | 9.3         | 7.5                          | 7.9                                               |
| 18101 | USA     | 45.7                  | 19.6                        | 39.2                           | 79.4                    | 1.7                               | 2.6                              | 69.3                | 30.7                  | 0.83                    | 0.90                    | 3.0                         | 82.4                    | 7.7                     | 8.6                    | 7.3                         | 9.4         | 7.6                          | 8.0                                               |
| 18201 | USA     | 47.0                  | 20.4                        | 40.0                           | 80.2                    | 1.8                               | 2.7                              | 70.1                | 29.9                  | 0.84                    | 0.91                    | 3.1                         | 83.2                    | 7.8                     | 8.7                    | 7.4                         | 9.5         | 7.7                          | 8.1                                               |
| 18301 | USA     | 48.3                  | 21.2                        | 40.8                           | 81.0                    | 1.9                               | 2.8                              | 70.9                | 29.1                  | 0.85                    | 0.92                    | 3.2                         | 84.0                    | 7.9                     | 8.8                    | 7.5                         | 9.6         | 7.8                          | 8.2                                               |
| 18401 | USA     | 49.6                  | 22.0                        | 41.6                           | 81.8                    | 2.0                               | 2.9                              | 71.7                | 28.3                  | 0.86                    | 0.93                    | 3.3                         | 84.8                    | 8.0                     | 8.9                    | 7.6                         | 9.7         | 7.9                          | 8.3                                               |
| 18501 | USA     | 50.9                  | 22.8                        | 42.4                           | 82.6                    | 2.1                               | 3.0                              | 72.5                | 27.5                  | 0.87                    | 0.94                    | 3.4                         | 85.6                    | 8.1                     | 9.0                    | 7.7                         | 9.8         | 8.0                          | 8.4                                               |
| 18601 | USA     | 52.2                  | 23.6                        | 43.2                           | 83.4                    | 2.2                               | 3.1                              | 73.3                | 26.7                  | 0.88                    | 0.95                    | 3.5                         | 86.4                    | 8.2                     | 9.1                    | 7.8                         | 9.9         | 8.1                          | 8.5                                               |
| 18701 | USA     | 53.5                  | 24.4                        | 44.0                           | 84.2                    | 2.3                               | 3.2                              | 74.1                | 25.9                  | 0.89                    | 0.96                    | 3.6                         | 87.2                    | 8.3                     | 9.2                    | 7.9                         | 10.0        | 8.2                          | 8.6                                               |
| 18801 | USA     | 54.8                  | 25.2                        | 44.8                           | 85.0                    | 2.4                               | 3.3                              | 74.9                | 25.1                  | 0.90                    | 0.97                    | 3.7                         | 88.0                    | 8.4                     | 9.3                    | 8.0                         | 10.1        | 8.3                          | 8.7                                               |
| 18901 | USA     | 56.1                  | 26.0                        | 45.6                           | 85.8                    | 2.5                               | 3.4                              | 75.7                | 24.3                  | 0.91                    | 0.98                    | 3.8                         | 88.8                    | 8.5                     | 9.4                    | 8.1                         | 10.2        | 8.4                          | 8.8                                               |
| 19001 | USA     | 57.4                  | 26.8                        | 46.4                           | 86.6                    | 2.6                               | 3.5                              | 76.5                | 23.5                  | 0.92                    | 0.99                    | 3.9                         | 89.6                    | 8.6                     | 9.5                    | 8.2                         | 10.3        | 8.5                          | 8.9                                               |
| 19101 | USA     | 58.7                  | 27.6                        | 47.2                           | 87.4                    | 2.7                               | 3.6                              | 77.3                | 22.7                  | 0.93                    | 1.00                    | 4.0                         | 90.4                    | 8.7                     | 9.6                    | 8.3                         | 10.4        | 8.6                          | 9.0                                               |
| 19201 | USA     | 60.0                  | 28.4                        | 48.0                           | 88.2                    | 2.8                               | 3.7                              | 78.1                | 21.9                  | 0.94                    | 1.01                    | 4.1                         | 91.2                    | 8.8                     | 9.7                    | 8.4                         | 10.5        | 8.7                          | 9.1                                               |

Figure 15L

pHRKAD5.dad MER6R2

19301 AGAAGTAAATG GGCACACAAAT CTATATCTCA CAGGCTAAT TACATATCTT TTATGEM'AA TTTTATATGT CTATATGATTT ACACACAGAC GGGTAAATAT  
 19401 TCTTGATATC GCGTTTGTGA GATACATGTA ATGTAACTGA AATCTCTTAT ANATATACCA GATTACATAA GATTACATAA TGTATATATC CCGATTATAT  
 19501 GGTGTTCTGG GCGCCCAACG ATCTAGTCTG TATATTTTGA AGACAGAAC ACAGATCTTT CATACACAGT TTTGCTTGAT TTTGCTTGAT TTTGCTTGAT  
 19601 GCGACAGACC GCGCGGTTCG TATGTCAGAC ATT'TAAATCT TCTGTCTTGG TGTCTCTGAA GTATGATGGA AATGCACTAT TTTGCTTGAT TTTGCTTGAT  
 19701 ATAGAACCAAG GTACTTTTCT ATTTGATATC AGCTATATAT CTACATATTA GATATATATTA AATATATATTA TTTAGTATAT TTTAGTATAT TTTAGTATAT  
 19801 TATCTTCTCT CATGAAAGGA TACAGCTTAG TCGACACACT GTTATATATTA GTTATATATTA GTTATATATTA GTTATATATTA GTTATATATTA GTTATATATTA  
 19901 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20001 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20101 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20201 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20301 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20401 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20501 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20601 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20701 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20801 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA  
 20901 TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA TTTTATATTA

Figure 15M

ДМРКАДСГАД МЕР6П2

[illegible]

Figure 15N







## pHRKadTag HIR682

|       |                                                                                                                                                                                                              |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 25701 | GGGCGCTTTC TCCAGAGAT GGCACCTAA AAGAACCTTC AATTTATTC GATACCTCAG GACAGAGATG AATACCTGGA CATCTGGA GATACAGTTT<br>CCCGGAGCG AGCGTCTTA CCGTGTAT TTCTTGAG TTACTGAGG TATATGAGG CTGCTCTCC TTATGAGCT GTACGTGCT GTCTCCAN |
| 25801 | TGACAGAGGA GAGGAGGAG ATGATGAGG ACTGAGAGAG GTTAAAGAG GAGCTCTCC GAGCTCTCC GAGCTCTCC GAGCTCTCC<br>ACCTGCTCTT TACTGCTCTT TGAGCTCTT GAGCTCTT GAGCTCTT GAGCTCTT GAGCTCTT GAGCTCTT GAGCTCTT                         |
| 25901 | CGCATTTCCC TCGGCGGCG CCGAGAGAT GCGAGAGAT TCGAGAGAT GCGAGAGAT GCGAGAGAT GCGAGAGAT GCGAGAGAT<br>CGCATTTCCC TCGGCGGCG CCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT                     |
| 26001 | AACCTAGAT GGCAGAGAG GGCAGAGAG GGCAGAGAG GGCAGAGAG GGCAGAGAG GGCAGAGAG GGCAGAGAG GGCAGAGAG<br>TTGCACTTA CCGTGTGAG ACCCTGCTT CCGCTATTA CCGCTATTA CCGCTATTA CCGCTATTA CCGCTATTA CCGCTATTA                       |
| 26101 | GGGCGCTTTC TCCAGAGAT GGCACCTAA AAGAACCTTC AATTTATTC GATACCTCAG GACAGAGATG AATACCTGGA CATCTGGA GATACAGTTT<br>CCCGGAGCG AGCGTCTTA CCGTGTAT TTCTTGAG TTACTGAGG TATATGAGG CTGCTCTCC TTATGAGCT GTACGTGCT GTCTCCAN |
| 26201 | GGGCGCTTTC TCCAGAGAT GGCACCTAA AAGAACCTTC AATTTATTC GATACCTCAG GACAGAGATG AATACCTGGA CATCTGGA GATACAGTTT<br>CCCGGAGCG AGCGTCTTA CCGTGTAT TTCTTGAG TTACTGAGG TATATGAGG CTGCTCTCC TTATGAGCT GTACGTGCT GTCTCCAN |
| 26301 | TACGAGAGT CTGAGAGAG CACAGAGAG CACAGAGAG CACAGAGAG CACAGAGAG CACAGAGAG CACAGAGAG CACAGAGAG<br>ATCGCTTGA GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC                       |
| 26401 | CTTAAAGCA GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT<br>GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT                               |
| 26501 | CCCGGAGCG AGCGTCTTA CCGTGTAT TTCTTGAG TTACTGAGG TATATGAGG CTGCTCTCC TTATGAGCT GTACGTGCT GTCTCCAN<br>GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC                          |
| 26601 | CTAGTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT<br>GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT                                        |
| 26701 | GATCAAGCG GCGGAGAG TTTAAATTC CCGCTGAT CCGCTGAT CCGCTGAT CCGCTGAT CCGCTGAT CCGCTGAT CCGCTGAT<br>GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT                              |
| 26801 | GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT<br>GATTTTTC CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT CACCTGAT                                         |
| 26901 | TCCCGGAGT TCGGCGGCG CCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT<br>ACCGGAGT TCGGCGGCG CCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT                        |
| 27001 | TCAGGAGCG AGCTTTCCT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT<br>AGCTTTCCT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT                       |
| 27101 | ACGAGAGAT GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC<br>TCTTGA GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC GAGCTGTTC                                    |
| 27201 | TCGAGAGAT TCGGCGGCG CCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT TCGAGAGAT<br>AGCTTTCCT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT CACAGAGAT                       |

Figure 150

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27301 CCGTCCCGCC ACTATCCGGA TCAATTTAT CTAACTCTTG ACATCTTAAA GCACTGTGCG GACCTCTAGC ACTGATGTTT AAGTGCAGAG CGAGACGAC  
 GGAAGGCGCG TGATAGGCGT AGTTANATTA GATTTGAGAC TGTATTTAT CTCTAGCCGC CTGCTGATGC TTACTTACAA TTCACTCTCT CATTCTCTT  
 27401 TCGCCCTGAA AGACTGTGTC CACTGTGTC GGCACACTG GCTTTCTGCG GACTTCGGTG ATTTTCTGTA CTTTGAATTT GCGGAGATAT ATATCTGATG  
 AGCGGACTT TGTGACACAG GTGACATCG CGGTGTTTAC GAATTTGCG CTGAGCGGCA TCAAAAGAT GAAACTTATC CGGCTCTAGC TATAGCTCT  
 27501 CCGCGCGCAC GCGTCTCGGC TTACCGCCCA GCGAGACTT GCGCTTATGC TGAATCGGTA GATTAACCGA CCGCCCTCTC TAGTTGTGCG GACAGCGTA  
 GCGCCCGCTG CCGCAGCGCG AATCGCGGCT CCGTCTCGAA CCGCATCGG ACTAAGCGCT GAAATGGGTC CCGGCGGAGG ATCAACTGCG CCGTCTCGCT  
 27601 CCGTGTGTTT TCACTGTGAT TTGCACACT TTGCACACTT CCAAGCCCTG GATTAACATCA AGATCTTTGT TCGCATCTCT TANTAAATAC AGAATTTAA  
 GGAACACAG ACTGACACTA AACGTTGACA GGAATTTGCA CCAATTTAGT TCTAGAAACA ACGGTATAGA CACGACTCAT ATATTTATG TCTTTAAT  
 27701 ATATCTGCG GCTCTGATCG CCACTCTGTA AACTCCAGCG TCTTACCGCG CCAAGCGCAA GGAATTTGAC CCAATTTGAC TACTCTCTC  
 TATATGACCG CAGGATATAG GTATGACAT TTGCGGTGCG AGATGTGCG GGGTCTCTTT GGTTCGCTT GGAATGAGC AACACCAAGC TCGAGAGCTA  
 27801 CTGTGATTTA CAACAGTTTC AACCGAGCG GATTAAGTCT ACCAGAGAC CACTCTCGGC GTCTCTCTG GAGAGCGCTG AOTCGATGAG GTAGTCTTTT  
 GACACTAAAT GTTGTCAAG TTGCGTCTCG CTACTGAGA TCGTCTCTG CACTCTCGGC ACCGTAAACC AGACTTTTC CCGACAGAGC TCAATTAATC TGTTTACCA  
 27901 CCGGTAACCT ACAGTGTGCT CAGCGCGCG GTGCGCGCG ACOTGTGCG GATGCGGAC TTGCAATTTG GTTTATGAG GATTAACCGA ACTGACCGG CTAATCTAT  
 GCGCTCTGCA TGTCTACGCA GTGCGCGCG ACOTGTGCG GTATTTAGCG AATGCGGAG CTACTGTGTA GATTAACCG GATTAACCG TCAATCTAT  
 28001 AACAGAGCT GAGCTTGAIA AACCTTTAG GTATTTAGCG AATGCGGAG CTACTGTGTA GATTAACCG GATTAACCG TCAATCTAT  
 TTGCTCTGCA CTGGAATCT TTGGAATCT CTAATATCG TTGCGCGCG GATTAACCG GATTAACCG TCAATCTAT  
 28101 TCAAGTTTCT CTGGAATCG GCTTGTGCT ATTCTGTG TTGGAATCT CTAATATCT ATACTAAGC TTCTCTGCT AAGCTCTGC GCTCTCTC  
 AGTCCAAAGA GATCTTAAGC CCAACCGCAA TAAGAGAGCG AACACTAGA GAATTAAGAA TATGATTTG AGACTAAGC TCCGAGCG TCCAGCTCTC  
 28201 TCGKCATTTG CATTTATTT CAGCTTTTGA AACGCTGCG TCGCACCCA AGATGATTA GATTAATATC CTAGCTTAC CTAGCTTAC GTCAGCTCTC  
 ACOTTAAGC GTAAATACCA GTGGAATAT TTGCGCGCG AGCGTGTG TCTACTAATC CATGATTTAG GATTAATATC AGTCCAAAGC AGTCCGCTC  
 28301 GGTACCAGCC AAGAGGTGIA TTTAAGGAG CCAAGCTGTA AGTTTACAT CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA  
 CCAAGCTGCG TTTCACCT AATATCTCT GGTGAGCAT TCAATATGTA CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA CCAAGCTGTA  
 28401 ATGAAAGCT CATTATTC CACAAACCA AATTTGCAA GTATCTGTT TATGCTATTT TCGAGCGCG TCAACTACA GATTAATATC TCAAGCTGTA  
 TACTTTTCCA CCAATAGCG GTGTTTTTGT TTAAAGCTT CATACGACA ATACGATTA CCGTCTGCT CCGTCTGCT CCGTCTGCT CCGTCTGCT  
 28501 CCAAGGTAAA AATCAFAAA CTTTATGTA TACTTTTCCA TTATATGAA TGTGAGAT TCACTATGAC ATGAGGAAAC AGTATAGCT GTGCGCGCA  
 GGTGCGCATTT TCAATATTT GAAATATCAT ATCAAAAGCT AATATCTTT ACAGCTGTA ATGATATG TACTCTGTT TCAATATCA CCGCTGCT  
 28601 CAATATGTTG TCGAAACAC TCGGCTTTC TGTGACTG CTATCTAT TACAGTCTC GCTTTGCTG GTAGCTCT CTATATTA TCAATATCA  
 GTTTTACAC ACCCTTTG ACCGTAAG ACGAGTGC GATACGATTA ATGTCAGAG CCAACGCA GATGATTA GATTAATTT ATGTTTCT  
 28701 GACGAGCTT TATTGAGIA AAGAAATC CTTAATTTAC TATGTTACA ACTAATTC ACCACTACT CCTTACTCG CCGTCTGCA ACAAATTT  
 CCGCTGCA ATACTCTT TTCTTTTAC GATTAATAT ATTCAATCT TCGATTAAG TGTGATTA CCAATATGAG GATTAATGAG TGTGATTA  
 28801 AAGGTTAGC ATTAATTA GATAGAT TAAACCGCG CCGTATTT TCTGATTA TCTGATTA TCTGATTA TCTGATTA TCTGATTA  
 TTTCATATC TAAATATAT CTATCTTAA ATTTGCGCG CCGTATGAG ACGATTTAG GATTAATGAG TCTGATTA TCTGATTA TCTGATTA

Figure 1SR

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28901 GGCCTACAC CTTGAGTCA GCTTCTCTG ATGTAGCAT CT ACTTGG CCAAGACTG TCCCTGGAT TCTTCCNGT CCACTACAG CCAACCACTC  
 CCGCATCTG GAATCTCACT CCAAGATC TACAGTCTA CA TGAACC GATGCTGAC AGGCTGCTA ANCAAGCTCA GCTTGGATG GCTTGGATG  
 29001 TACAGGAT GACCAACCA ACCACAGCG CCGCTCTAC CCACTTACA TTATCCACA ATATCCGCA TATCTGCTC AGTTCTGCC TTTTCAATA ATGAGTAA  
 ATTCTCTA CTGCTCTG TCTTCTGCT CCACTCTG GCTTCTGCT AGATCTGCT TATCTGCTC TCAAGACAG AACACTTAT TCACTCTAT TCACTCTAT  
 29101 CTGCTCTG TCTTCTGCT CCACTCTG TATCTCTA TATCTCTG CACTCTGCT CTAAGCTCA ACCGCTGCT TCAAGCTCA ACCGCTGCT TCAAGCTCA  
 GACCTCTG ACCACCAAG GATCTCTG ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT  
 29201 TATCTCTG TCACTCTG ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT ATACCAAT  
 ATATCTGCT AGTACCTG TCTTCTGCT TACTCTGCT TACTCTGCT TACTCTGCT TACTCTGCT TACTCTGCT TACTCTGCT TACTCTGCT  
 29301 CACTCTGCT CCACTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG  
 TACTCTGCT CCACTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG TATCTCTG  
 29401 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 29501 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 29601 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 29701 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 29801 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 29901 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 30001 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
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 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 30201 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 30301 GCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT  
 CCACTCTG TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT TCTTCTGCT

Figure 155

pMIRKad5-qmg HER682

30401 AATTTCTCTG CAGTGTATAT CAGACGACC TCTTGTGCTT CTTCTCACTT CTCTATTTTC AGCTTCCTCC CTTCTCCAA TGTCTCCAA CTTCTCCAC ATCTAAATG  
 TTTAAAGACA GTTCATATTA GTCTGTGTG AGTAAACGGA GTATGTATGA GATATATAGG TGTATATAGG GAAGAGGTG GAAGAGGTG TTAGATTAC  
 30501 GAATGTACGT TCTCTCTGT TCTGTGCAAT CCGTACCCAC TATCTTCATG TTTTGTATGA TGTATGTGTC TGAAGTGTG TGAAGTGTG TGAAGTGTG TGAAGTGTG  
 CTTACACTCA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA AGCAAGACGA  
 30601 GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 CATAGGTATA CTGTGTCTTT CCGTACCCAC TGTATGTATAT TGTATGTATAT TGTATGTATAT TGTATGTATAT TGTATGTATAT TGTATGTATAT TGTATGTATAT TGTATGTATAT  
 30701 TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT TCTTGTGCTT  
 AGAAGCCGCG ATAGCTTTGG AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT  
 30801 AATATGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 TTTTACATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 30901 GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 CCGACGCGCG GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 31001 GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 31101 TAACTACTTC CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 ATTATATATGT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT GTATGTATAT  
 31201 AGAAGCCGCG ATAGCTTTGG AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT  
 TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT  
 31301 CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT  
 31401 AATATATATGT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT AGTATGTATAT  
 TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT TCTGTGTATAT  
 31501 GAATGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT GTTGTGTATAT  
 31601 TCACTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 AUTGTATATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 31701 TTAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT AATATATATGT  
 31801 TCACTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 ACCTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 31901 ATATGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT TATATGTATAT  
 32001 GAATGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT CAGTGTATAT  
 CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT CTTTGTATAT

Figure 15T

pHKKAD5959g MFE6682

32101 TAACTATGTC AGTCAGATTT ACTTAAACCG AGCAAAAGCT AAKKCTTA CACTAACCT TACTACTAAC GGTACACAGG AATACAGAGA CACACTCTA  
 ATTGTAAAGG TCGATTCAAA TGATTTTTCG TCTCTTTTGA TTTTACACT GTGATTTTGG CCATGTGTGC TTGTGTCTCT TTGTGTCTCT GTGTGTGTCT  
 32201 AGTGCATCT CTATGTCTAT TTCTATGTC AGCAATACAT TATCTAAATA TTCTCTACAT TTCTCTACAT TTCTCTACAT TTCTCTACAT TTCTCTACAT  
 TCACTATAGA CATACAGTAA AGTACCTCG ATCAACACCG TCTCTATTTA TATCTCTAT TATCTCTAT TATCTCTAT TATCTCTAT TATCTCTAT  
 32301 AATAAGAAAT GCTTTGTGTT ATGTCTTAC ATCTCTTAC CACAAATTA AATTTAACT TCTTTAACT TCTTTAACT TCTTTAACT TCTTTAACT  
 TTTTCTCTTA GCAACACAA TACAACTTG CACAACTTA CACAACTTA CACAACTTA CACAACTTA CACAACTTA CACAACTTA CACAACTTA  
 32401 GCTTATACAG ATCACTCTAC CTTAACTTAA GATTTTCTTG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG  
 CCAATATATC TATGTGTGAG GAATTTTCTT GATTTTCTTG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG CACTATTAAG  
 32501 GCTGTCTCTA AATGCACTCA TATCACTCT TATCACTCT TATCACTCT TATCACTCT TATCACTCT TATCACTCT TATCACTCT TATCACTCT  
 CCACTCTCTA TTTCTCTAGT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 32601 ATAACTCTCT CCACTCTCTC ACTTATCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 TATTTTCTCT CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 32701 AATCTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 TTCTCTCTCT CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 32801 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 GCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 32901 TCACTCTCTA CCACTCTCTA ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 AGTCACTCTA CCACTCTCTA ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33001 AATCTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 TTCTCTCTCT CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33101 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 GTCTCTCTCT CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33201 AATCTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 TCTCTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33301 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33401 AATCTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 TTCTCTCTCT CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 33501 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT  
 CCACTCTCTC CCACTCTCTC ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT ATCTCTCTCT

Figure 15U

pHRKad5gag MER682

33601 CTGAGACAAA ACCAGGTGCG GCGGTACAAA ACAGATCTGC GTCTCTGCTG TTTGCGTCTA GATGCTCTCG TGTAGTAGTT GTATGATATC CACTCTCTTA  
 GACTTCCTTT TGGTCCAGGC CCGCATTTT TGTCTAGACG CAGAGCTGTC CAGAGCTGTC AGTGGGAT ACATCATCAA CATCATATAG CTGAGAGATT  
 33701 AAGCATCCAG CCGCCCGCTG GCTTCGGTCT CTATGTAAAC TCTCTATGTC TCTCTATGTC CCGCTGCTCC TATATACATC CACCACCGCA GAAATAGCCA CACCCAGCC/  
 TTCTATGCTC CCGCCCGGAC CCAAGCCCAA CATACATTTG AGTAAGTAGC CCGCTGCTCC AGTATTTGAG GTGGTGGGCT CTATTTGCTT GTGGTCTGCT  
 33801 ACCTACACAT TCGTCTGCGC AGTCACACAC CCGAGTACG GTAAAGTCTG GTAAAGTCTG GTAAAGTCTG GTAAAGTCTG GTAAAGTCTG GTAAAGTCTG  
 TCGATGTCTA AGCAAGACGC TCGATGTCTG CCGCTCTGCG CCGCTCTGCG CCGCTCTGCG CCGCTCTGCG CCGCTCTGCG CCGCTCTGCG CCGCTCTGCG  
 33901 ATGAGATCT ATTAAGTGAA CCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC  
 TACTCTAGTA TATTTCACTT CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG CCGGAGCGCG  
 34001 TCCAAAGCG AAGCGCGCT CAGGTCCAG TCGAGCTAAA GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC  
 AGGTTTTTCC TTGCGCGGGA GTGCGGCTTC ACTGCGATTT CCGATTTTCC AGTCCCACT TAGAGAGGAT ATTGTGAGCG TCGTGGAAAT TGGTACCGGT  
 34101 AATAATTC ATCTGCGAC CTCTCTAATA TATCTCTAAG CAAATCCGTA ATATTAAATC CCGGCTGCGC TCGGCTGCGC TCGGCTGCGC TCGGCTGCGC  
 TTATTAGAG TAGAGCGGTG GAAGAGTTAT ATAGAGTTAT ATAGAGTTAT ATAGAGTTAT ATAGAGTTAT ATAGAGTTAT ATAGAGTTAT ATAGAGTTAT  
 34201 CAGCTCTAG CAGCTCTAG TGAATGAAA AATTCAGGTT CCGTACAGC CTGTATAGTA TTTTAAAGCG CCGGCTGCGC TCGGCTGCGC TCGGCTGCGC  
 GTGCGAGTTC GTGCGAGTTC ACTGAGTTC TTAAGTCCAA GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC GGTAAAGCGC  
 34301 GTGCGGCTG CAGGCGCAG TGAACATAT GTGCGGCTG CAGGCGCAG TGAACATAT GTGCGGCTG CAGGCGCAG TGAACATAT GTGCGGCTG  
 CAGGCGCAG GTGCGGCTG ACTGCTATTA GCACTCTCAG CAGGCGCAG TGAACATAT GTGCGGCTG CAGGCGCAG TGAACATAT GTGCGGCTG  
 34401 TATGAGCGC ATACTGAGG CTATCTAAC CAGGCTGCGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC  
 ATACTGAGG TATGAGCGC GATAGGATTC GTGCGGCTG CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC  
 34501 GGCAGAGCT GGCAGAGCT AGAGAGCTA TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 CCGTTTCCGA CCGTTTCCGA TCTTCTGT AGATCTAGT CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC CCGATGAGC  
 34601 TCTCAACAT GTCTGCGGT TCTGCTATA ACAGAGTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 AGAGTTCTTA CAGAGCGCCA AGAGGTTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 34701 ATAGAGCTA GAGCGCTAC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC CCGGCTGCGC  
 TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT TATCTGAT  
 34801 TCAATATATA AGACTGCTA AACACATCAG GTTGAATCAG ATCTGCTAG CAGTCTAGT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 AGTATATCAT TCTGAGCGCT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 34901 AGCACAT TACAGCGGCA TAGAGGATAT AACAAATTA TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT TGTAGTCTAT  
 35001 TCCGCTGCTA GAGCAGCTA CAGGCTGCT CAGGCTGCT CAGGCTGCT CAGGCTGCT CAGGCTGCT CAGGCTGCT CAGGCTGCT CAGGCTGCT  
 AGGCGAGCT CTGTTGATAT GTGCGGAGG TGTGCGGCT TGTGCGGCT TGTGCGGCT TGTGCGGCT TGTGCGGCT TGTGCGGCT TGTGCGGCT  
 35101 GGCAGAGCT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT CAGTCTAGT  
 CCGTGGCTA GTTATGCT GTTATGCT GTTATGCT GTTATGCT GTTATGCT GTTATGCT GTTATGCT GTTATGCT GTTATGCT  
 35201 CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT CCGAGAGCT  
 GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT GGTGCTGCT

Figure 15V





pmrkad5seq MER682

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37001  CACACCGGGA TAAATACCGCG CCACATATCA CAACCTTAAA AGTGTCTCATC ATTGGAAGAC GTTCTTCGGG GCGAAUACAC TCAGGGAATC TACTTCTCTT
GTGTGCGCCT ATTATCGCGC GGTATCGCGC GGTGTATCGT CTTCGAATTT TACAGCTAG TACCTTTTG CAGAGCGCC CCGTTTGGAG AGTCTCTTCA ATGCTGACAA
37101  GAGATCCAGT TCGATGTAC CCACTCGTGC GGTGTATCGT ACCTAACCTA TCTTAACTAT CTTTAACTTT CACCTACCTT TCTGCGGTAG CAAAGACAGG AAGCGCAAAAT
CTCTAGGTCA AGCTACATTC GGTGACGACG TCGGTGACT TCGGTGACT AGAATGCTTA GAAATGAAA GTTCTGCTTA AGACCCACTC GTTTTGTCC TTCCCTTTTA
37201  GCGCGAAGAA AGGGAATAG GCGGACAGCG AATGTGTAAA TACTATATCT TTCTCTTTT CAAATATAT GTAGCATTTA TCAGGTATAT TCTCTCATCA
CGCGCTTTT TCCTTATTC CCGCTGAGC TTTCACACTT ATGACTATATCA GAGGGAAGAA GTTATATATA CTTCGTAAAT AGTCCCAATA ACAGAGTATC
37301  GCGGATACAT ATTGGAATGT ATTAGAAA ATTAAACAAAT AGGATGTTTG CAGATATTC CCGGAAATGT GCGACCTGAC GTCTAAGAAA CCAATTATTA
CGCTATGTA TAACTTTACA TAAATCTTTT TATTGTGTTA TCCCGAAGCG CCGTGTAAAG GCGCTTTTCA CCGTGGACTG CAGATCTCTT GGTATATATA

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Band 5  
Figure 15X

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37401  CATGACATTA ACCTATAAA ATAGCGTAT CACTAGCGCC TTCTCTTTC AGAATTTGA TTCTGATTTCT TAAT (SEQ ID NO: 27)
GTACTGTAT TCGATATTTT TATCCGATA GGTCTCGCG GAGCGAGAG TTCTTAACT AGCTTAAGA ATTA (SEQ ID NO: 28)

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Figure 15X

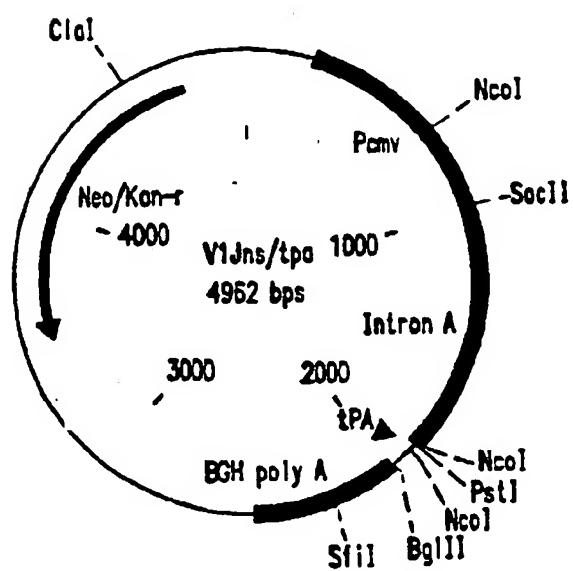
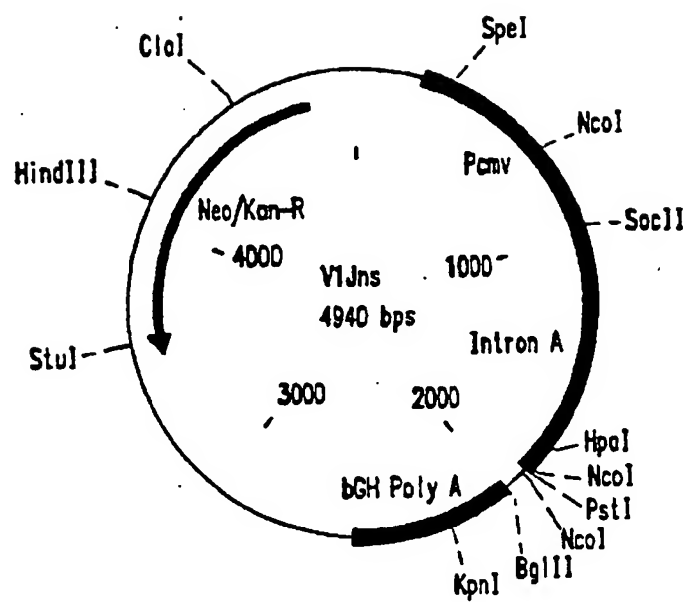


FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCATTGAGACTGTCCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGTGAA  
 Bg/II MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLy  
 1 10 20  
 GCAGTGGCCCCCTGACTGAGGAGAAGATCAAGCCCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA  
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL  
 30 40 50  
 AGATTGGCCCCGAGAAGCCCTACACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG  
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal  
 60 70  
 GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA  
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy  
 80 90 100  
 GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCACTTCTCTGTGCCCTGGATGAGGACTTCAGGAAGTACACTG  
 slsLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA  
 110 120 130  
 CCTTCACCATCCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC  
 loPheTnrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly  
 140 150  
 TCCCCTGCCATCTTCCAGTCTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTTGTATCTACCA  
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGl  
 160 170 180  
 GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCAAC  
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL  
 190 200 210  
 TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGAGCTGCAC  
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis  
 220 230  
 CCGACAAGTGGACTGTGCAGCCCATTTGCTGCTGAGAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG  
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGl  
 240 250 260  
 CAAGCTGAAGTGGGCTCCCAAATCTACCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC  
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL  
 270 280 290

FIGURE 17A

TGA CTGAGG TGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGCAGATCCTGAAGGAGCCTGTGCAT  
 EuThrGluVolIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProVolHis  
 300 310

GGGGTGTA CTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAATCTA  
 GlyVolTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy  
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGCCACACCAATGATGTGAAGCAGCTGA  
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspVolLysGlnLeuT  
 350 360 370

CTGAGGCTGTGCAGAGATCACCAGTGGTCCATTGTGATCTGGGCAAGACCCCAAGTTCAAGCTGCCATCCAGAAG  
 hrGluAlaVolGlnLysIleThrThrGluSerIleVolIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys  
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT  
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheVolAsnThrProProLe  
 400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCATTGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG  
 uVolLysLeuTrpTyrGlnLeuGluLysGluProIleVolGlyAlaGluThrPheTyrVolAlaGlyAlaAlaAsnArgG  
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGCAGGCAGAGGTTGGTGACCTGACTGACACCACCAACCCAG  
 luThrLysLeuGlyLysAlaGlyTyrVolThrAsnArgGlyArgGlnLysVolVolThrLeuThrAspThrThrAsnGln  
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC  
 LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluVolAsnIleVolThrAlaSerGlnTyrAl  
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG  
 aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuVolAsnGlnIleIleGluGlnLeuIleLysLysG  
 510 520 530

AGAAGGTGTACCTGGCTGGCTGGCTGCCACAAGGCCATTGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC  
 luLysVolTyrLeuAlaTrpVolProAlaHisLysGlyIleGlyGlyAsnGluGlnVolAspLysLeuVolSerAlaGly  
 540 550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGCCCCAGGATGAGCATGAGAAGTACCACTCCAAGTGGAGGGCTAT  
 IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe  
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCTGTGGTGGCTAAGGAGATTGTGGCTCCCTGTGACAAGTGCACGTGAAGGGGAGG  
 tAlaSerAspPheAsnLeuProProVolVolAlaLysGluIleVolAlaSerCysAspLysCysGlnLeuLysGlyGluA  
 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG  
 lAlaMetHisGlyGlnVolAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysVolIleLeuVol  
 620 630

GCTGTGCAATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCTGCT  
 AlaVolHisVolAlaSerGlyTyrIleGluAlaGluVolIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe  
 640 650 660

GAAGCTGGCTGGCAGGTGGCTGTGAAGACCATCCACACTGCCAATGGCTCCAACCTCACTGGGGCCACAGTGAGGGCTG  
 uLysLeuAlaGlyArgTrpProVolLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrVolArgAlaA  
 670 680 690

CCTGCTGGTGGCTGGCATCAAGCAGGAGTGTGGCACTCCCTACAACCCCACTCCAGGGGTGGTGGCTCCATGAAC  
 lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyVolVolAlaSerMetAsn  
 700 710

AAGGAGCTGAAGAAGATCATTTGGCAGGTGAGGGACCAGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTTAT  
 LysGluLeuLysLysIleIleGlyGlnVolArgAspGlnAlaGluHisLeuLysThrAlaVolGlnMetAlaVolPheIle  
 720 730 740

CCACAACCTCAAGAGGAAGGGGGCATCGGGGCTACTCCGCTGGGAGAGGATTGTGGACATCATGCCACAGACATCC  
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleVolAspIleIleAlaThrAspIleG  
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGACTCCAGGAACCCCTGTGG  
 lThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgVolTyrTyrArgAspSerArgAsnProLeuTrp  
 780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGAGGGGGCTGTGGTGATCCAGGACAACCTCTGACATCAAGGTGGTGGCCAG  
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaVolVolIleGlnAspAsnSerAspIleLysVolVolProAr  
 800 810 820

GAGGAAGGCCAAGATCATCAGGACTATGGCAAGCAGATGGCTGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT  
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx  
 830 840 850

AAAGCCCGGCAGATCT (SEQ ID NO: 3)  
 Xx BgllI (SEQ ID NO: 4)

FIGURE 17C

**FIGURE 18**

|     |                                                                                          |      |
|-----|------------------------------------------------------------------------------------------|------|
| WT  | - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT                                | -42  |
| OPT | - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC<br>M G G K W S K R S V P G W S | -14  |
| WT  | - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT                                | -84  |
| OPT | - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC<br>T V R E R M R R A E P A A D | -28  |
| WT  | - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA                                | -126 |
| OPT | - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC<br>R V R R T E P A A V G V G A | -42  |
| WT  | - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC                                | -168 |
| OPT | - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC<br>V S R D L E K H G A I T S S | -56  |
| WT  | - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA                                | -210 |
| OPT | - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC<br>N T A A T N A D C A W L E A | -70  |
| WT  | - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA                                | -252 |
| OPT | - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG<br>Q E D E E V G F P V R P Q V | -84  |
| WT  | - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC                                | -294 |
| OPT | - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC<br>P L R P M T Y K G A V D L S | -98  |
| WT  | - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC                                | -336 |
| OPT | - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC<br>H F L K E K G G L E G L I H | -112 |
| WT  | - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC                                | -378 |
| OPT | - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC<br>S Q K R Q D I L D L W V Y H | -126 |
| WT  | - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG                                | -420 |
| OPT | - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC<br>T Q G Y F P D W Q N Y T P G | -140 |

FIGURE 19A

|     |                                                              |      |
|-----|--------------------------------------------------------------|------|
| WT  | - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG    | -462 |
|     |                                                              |      |
| OPT | - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG    |      |
|     | P G I R F P L T F G W C F K                                  | -154 |
| WT  | - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA    | -504 |
|     |                                                              |      |
| OPT | - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG    |      |
|     | L V P V E P E K V E E A N E                                  | -168 |
| WT  | - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG    | -546 |
|     |                                                              |      |
| OPT | - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC    |      |
|     | G E N N C L L H P M S Q H G                                  | -182 |
| WT  | - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC    | -588 |
|     |                                                              |      |
| OPT | - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC    |      |
|     | I E D P E K E V L E W R F D                                  | -196 |
| WT  | - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG    | -630 |
|     |                                                              |      |
| OPT | - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC    |      |
|     | S K L A F H H V A R E L H P                                  | -210 |
| WT  | - GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)                 | -651 |
|     |                                                              |      |
| OPT | - GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9) |      |
|     | E Y Y K D C (SEQ ID NO:10)                                   | -216 |

FIGURE 19B



VIJns/nef *PstI* *BglII*  
 CATGGGTCCTTTCTGAGTACCGTCCCTTGAATGTCGCCACC ATG GGC GGC AAG TGG TCC MAG AGG TCC GTG CCC  
 M G G K W S K R S V P  
 . . . . . CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGACAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 38)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 10) *SrfI* *BglII*

VIJns/nef(G2A, LLAA)  
*PstI* *BglII*  
 CATGGGTCCTTTCTGAGTACCGTCCCTTGAATGTCGCCACC ATG GGC GGC ANG TGG TCC AAG AGG TCC GTG CCC  
 M A G K W S K R S V P  
 . . . . . CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGACAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 39)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 14) *SrfI* *BglII*

VIJns/tpanef & VIJns/tpanef(LLAA)  
*PstI*  
 CATGGGTCCTTTCTGAGTACCGTCCCTTATATCTAGATCACC ATG GAT GCA ATG MAG AGA GGG CTC TGC TGT GTG  
 M D A M K R G L C V  
 CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ATC TCC TCC MAG AGG TCC GTG CCC  
 L L L C G A V F V S P S E I S S K R S V P  
 . . . . . CAC CCC GAG TAC TAC MAG GAC TGC TAA AGCCCGGACAGATCTGCTGTGCTCTTAGTTGCCAGC (SEQ ID NO: 40)  
 H P E Y Y K D C \* (contained within SEQ ID NO: 16) *SrfI* *BglII*

FIGURE 20

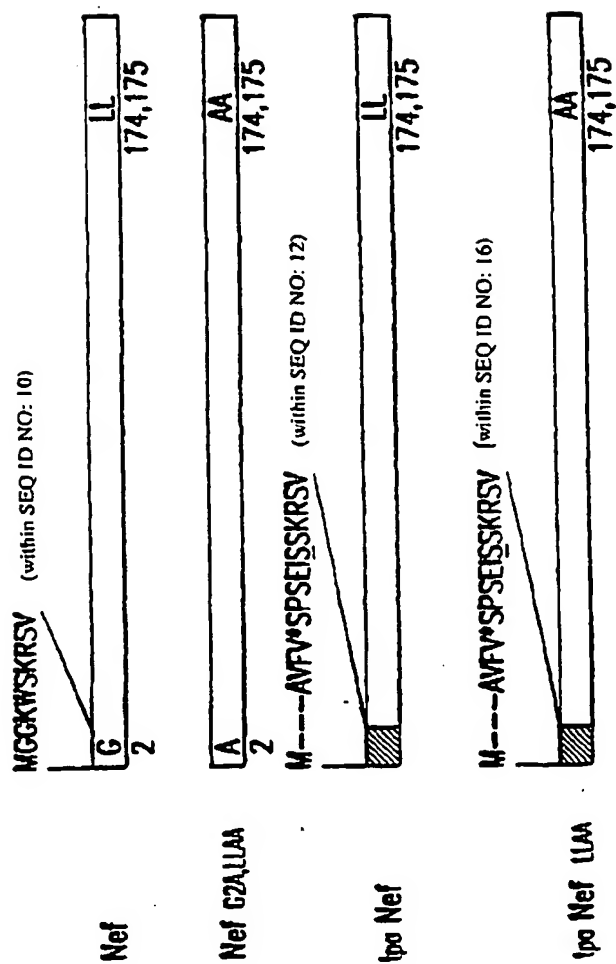


FIGURE 21

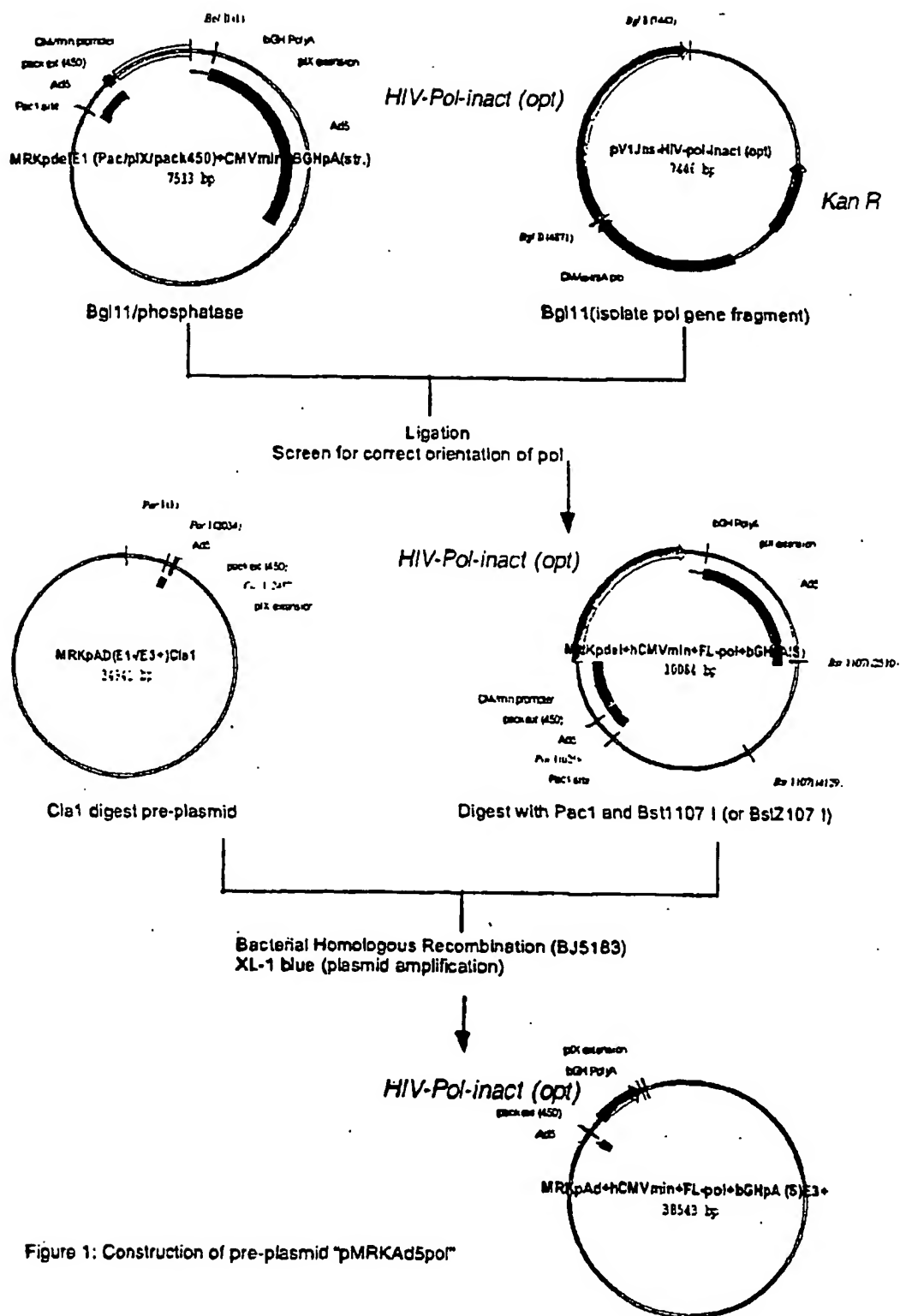
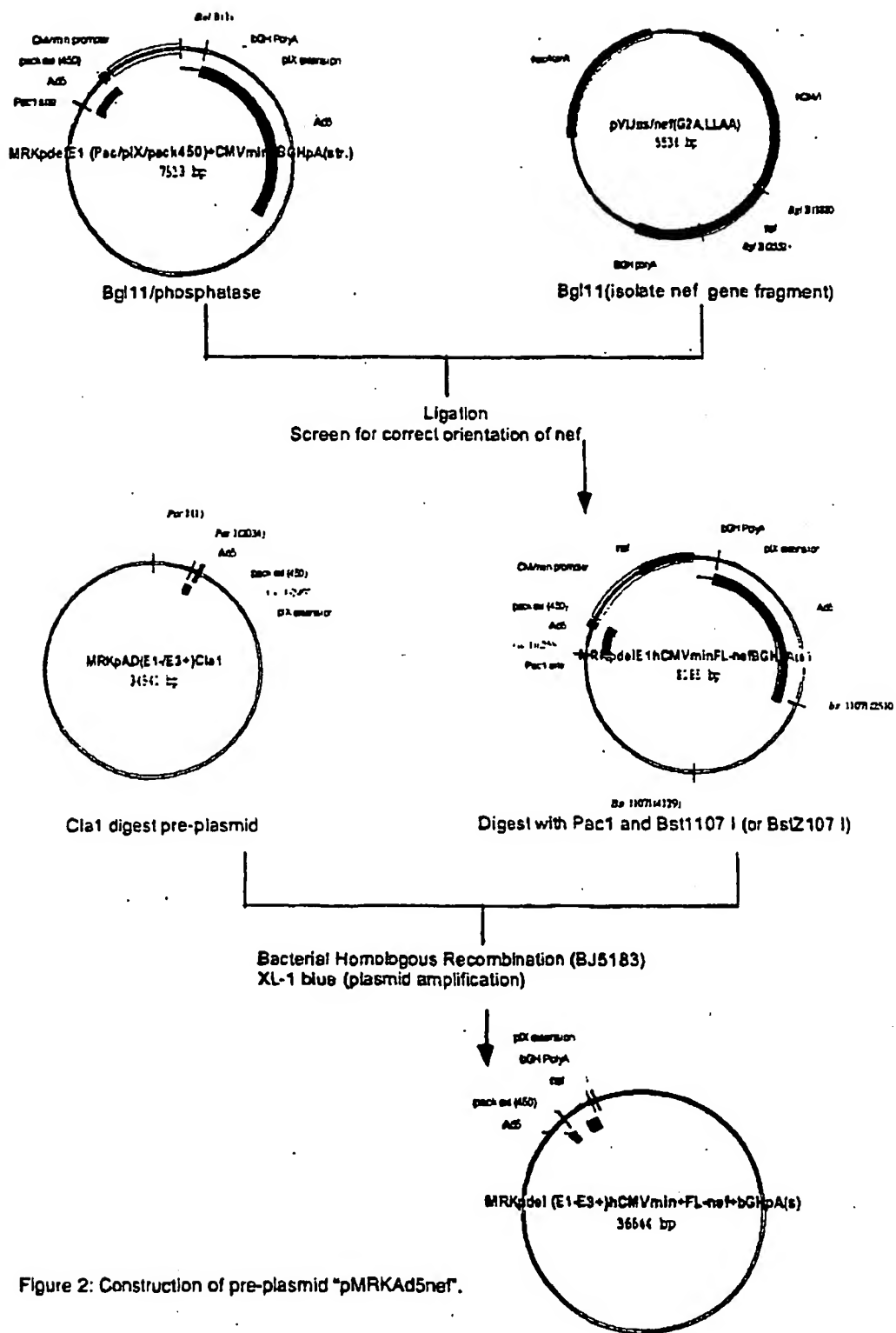


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

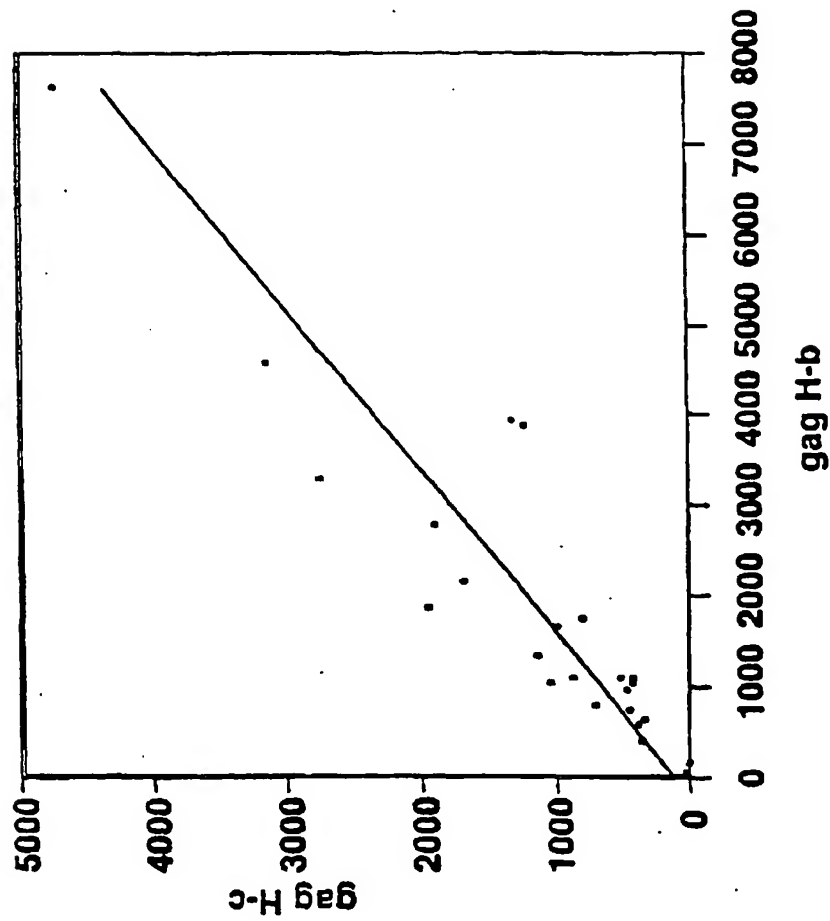
FIGURE 22



**Figure 2: Construction of pre-plasmid "pMRKAd5nef".**

**FIGURE 23**

# Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



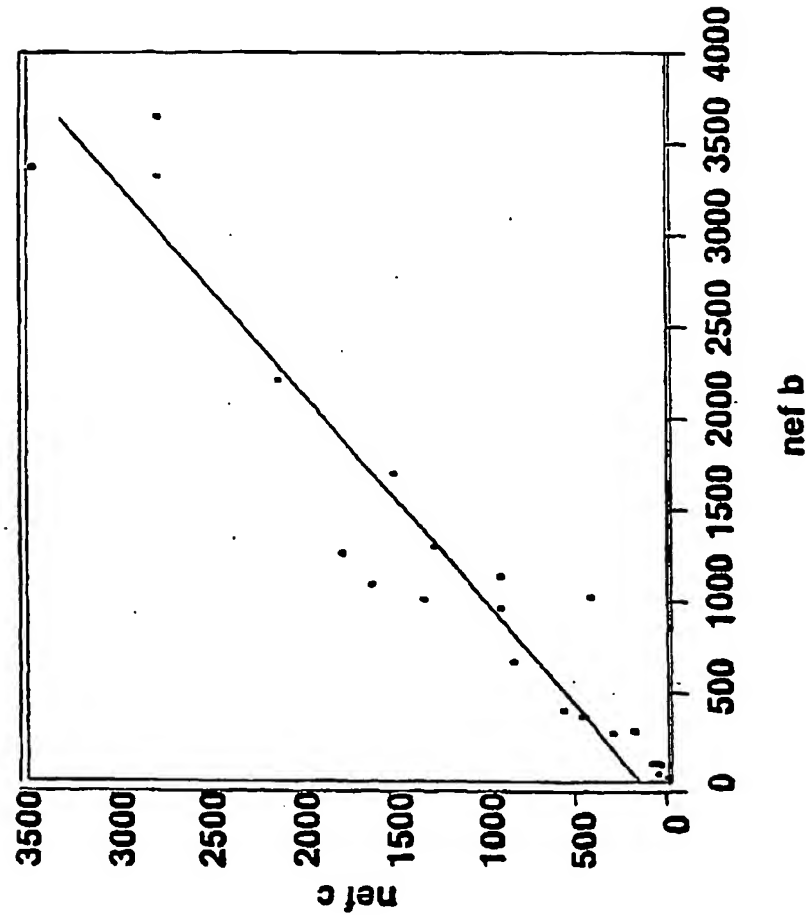
Linear Fit

$$\text{gag H-c} = 111.603 + 0.55866 \text{ gag H-b}$$

Summary of Fit

|                            |          |
|----------------------------|----------|
| RSquare                    | 0.816775 |
| RSquare Adj                | 0.80914  |
| Root Mean Square Error     | 474.9639 |
| Mean of Response           | 1158.115 |
| Observations (or Sum Wgts) | 26       |

# Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



nef c = 131.132 + 0.8646 nef b

## Summary of Fit

|                            |          |
|----------------------------|----------|
| RSquare                    | 0.91685  |
| RSquare Adj                | 0.91289  |
| Root Mean Square Error     | 289.7718 |
| Mean of Response           | 1086.435 |
| Observations (or Sum Wgts) | 23       |

FIGURE 25

**MRKAd5pol MER1062**  
**(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)**

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACCTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCGGCGCCCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCGCGGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCGGGCGCT AGSTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCGG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAACTGCCCC CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCASTA
   GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTAATTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

*Figure 26A*

901 TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC  
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG  
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG  
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG  
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG  
GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC  
CTCGACTTGT TCTCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG  
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG AACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT  
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTTCATGTA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA  
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT  
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT  
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 24B



1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG  
 CCCC GGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC  
 1951 TGGATGGGCT ATGAGCTGCA CCCCACAAAG TGGACTGTGC AGCCCATTGT  
 ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA  
 2001 GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG  
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC  
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC  
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT  
 GACACGTTCG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA  
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG  
 CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC  
 2201 AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG  
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC  
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC  
 TAGGTCTTCG TCCCGGTCCC GGTACCTGG ATGGTTTAGA TGGTCTCGG  
 2301 CTCAAGAAGC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCACAC  
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GGGTGT  
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
 GGTTACTACA CTTCGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC  
 2401 TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA  
 AGGTAACACT AGACCCCGTT CTGGGGGTTC AAGTTCGACG GGTAGGTCTT  
 2451 GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC  
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG  
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG  
 GACTCACCTT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC  
 2551 CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC  
 GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGAAGATAC ACCGACCCCG  
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG  
 ACGGTTGTCC CTCTGGTTCG ACCCGTTCCG ACCGATACAC TGGTTGTCCC  
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG  
 2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT  
 GAGGTCCGGT AGATGGACCG GGAGGTCCTG AGACCGGACC TCCACTTGTA  
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC  
 ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26C

2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA  
CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC  
ACTCGTCCAC CTGTTTCGACC ACAGACGACC GTAGTCCTTC CACGACAAGG

2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
ACCTACCGTA ACTGTTCCGG GTCTACTCG TACTCTTCAT GGTGAGGTTG

3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA  
ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT

3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG  
CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101 GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG  
CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA  
CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAC

3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC  
CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301 TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT  
AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG  
GTTCTCCTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC

3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG  
GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC

3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT  
CGACTCGTGG ACTTCTGTCTG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG  
GTTCTCCTC CCCCCGTAGC CCCCAGTAGG GCGACCCCTC TCCTAACACC

3551 ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
TGTAAGTACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG

3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG  
TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCTT TGGGGGACAC

3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC  
CTTCCCGGGA CGGTTCGACG ACACCTTCCC CCTCCCCCGA CACCACCTAGG

3701 AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC  
TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

Figure 26 D

3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCCTTCT AGTTGCCAGC  
CCTACTCCTG ATTTCTGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG

3851 CATCTGTTGT TTGCCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC  
GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACGGGA CCTTCCACGG

3901 ACTCCCCTG TCCTTTCCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT  
TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG  
CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTC

4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT  
CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051 ATGGCCGATC GGC GCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG  
TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCAC

4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC  
CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTGATGGA AGCATTGTGA  
TCGTCGGCGG CCGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAA  
CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAACTCTAC  
CACTACCCGA GGTCGTAACCT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT  
ATGGAACCTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351 CCGCCCGCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC  
GGCGCGGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC  
AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC  
GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501 GGGAACTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT  
CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTGCTCCAA

4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA  
AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTAT

4601 AAAACCAGAC TCTGTTTGGG TTTGGATCAA GCAAGTGTCT TGCTGTCTTT  
TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTCTG  
TAAATCCCA AAACGCGCGC GCCATCCGGG CCTGGTCTG CAGAGCCAGC

Figure 26E

4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCACCT  
CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA

4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG  
CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC  
CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG

4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTTA AGCTGGGATG  
GTCCCCGTCC GGAACACACA TTCACAAATG TTTCGCCAAT TCGACCCTAC

4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTAGGTTG  
CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC  
CGATACAAGG GTCGGTATAG GGAGGCCCTT AAGTACAACA CGTCTTGGTG

5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG  
GTCGTGTCAC ATAGGCCACG TGAACCTTTT AACAGTACA TCGAATCTTC

5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC  
CTTACGCAC CTTCTTGAAC CTCTGCGGGA AACTGGAGG TTCTAAAAGG

5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC  
TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCCGC GCCGGACCCG

5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT  
CTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251 CGTCATAGGC CATTTTACAA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT  
GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCACGG TCTGACGCCA

5301 ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT  
TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351 TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TGCGGGGCGA  
AAGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT

5401 TGAAGAAAAC GGTTCGCGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG  
ACTTCTTTTG CCAAAGGCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC

5451 TTCCTGAGCA GCTGCGACTT ACCGAGCCG GTGGGCCCCG AAATCACACC  
AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG

5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC  
ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC  
ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG  
GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26 F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC  
 AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG  
 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG  
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC  
 5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT  
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA  
 5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG  
 GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC  
 5901 TGAAGGGGTG CGCTCCGGGC TGC CGCTGG CCAGGGTGCG CTTGAGGCTG  
 ACTTCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC  
 5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG  
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGTC  
 6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT  
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACC GGGA  
 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA  
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT  
 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA  
 GAAAACCTCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCTCAT  
 6151 GGCATCCGCG CCGCAGGCC CCGCAGACGGT CTCGCATTCC ACGAGCCAGG  
 CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC  
 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCC ATGCTTTTTG  
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC  
 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC  
 TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG  
 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTCGA  
 CTTTTCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT  
 6351 GCGGTGTTCC GCGGTCTCTC TCGTATAGAA ACTCGGACCA CTCTGAGACA  
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT  
 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG  
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCAACC TCCCCATCGC  
 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT  
 CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA  
 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG  
 GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC  
 6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC  
 ACTGGCCAC AAGGACTTCC CCCCATATT TTCCCCACC CCCGCGCAAG

Figure 266

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT  
TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTC TAACAGTCAA

6701 TCCAAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT  
AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGA

6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA  
CTCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT

6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTGGCGATG  
CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC

6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT  
CTCGCGTCCC AAACCAAAAA CAGCGCTAGC GCGCGAGGA ACCGGCGCTA

6901 GTTTAGCTGC ACGTATTTCG GCGCAACGCA CCGCCATTTC GGAAAGACGG  
CAATCGACG TGCATAAGCG CGCGTTGCGT GCGGTAAGC CCTTCTGCC

6951 TGGTGCCTC GTCGGGACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG  
ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC

7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT  
CACTGTTCCA GTTGCACCA CCGATGGAGA GGCGCATCCG CGAGCAACCA

7051 CCAGCAGAGG CGGCCGCCCT TGC GCGAGCA GAATGGCGGT AGGGGGTCTA  
GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT

7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CCGTAAAGAC CCCGGGCAGC  
CGACGCAGAG CAGGCCCCC AGACGCAGGT GCCATTCTG GGGCCCGTCG

7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCACT CTTTGCAAGT CTAGCGCCTG  
TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC  
GACGGTACGC GCCCGCCGTT CCGCGCGGAG CATACCCAAC TCACCCCTG

7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTCG  
GGGTACCGTA CCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC

7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT  
ATTTGCATCT CCCCAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA

7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTTC TGCGAGGGAG  
AGGTGGCGCC TACGACCGCG CGTGCAATTAG CATATCAAGC ACGCTCCCTC

7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG  
GCTCCTCCAG CCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC

7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG  
TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG  
CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

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7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG
      AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC

7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT
      AGGGAAAAAA AAGGTGTCGA GCGCCAAC TC GTTTGAGA AGCGCCAGAA

7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
      AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCCTCGGA

7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
      TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG

7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG
      CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC

7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
      GTTCCACAG GGACTGGTAC TGAAACTCCA TGACCATAAA CTTCAGTCAC

7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA
      AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT

7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
      TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC

8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
      GCGCTCCGTA TTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT

8051 CGGTGTGTTAA TTACCTGGGC GGCGAGCAG ATCTCGTCAA AGCCGTTGAT
      GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA

8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
      CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAAGTACC

8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
      TTCCGTTAAA AAATCAAGG AGCATCCACT CGAGAAGTCC CTCGACTCG

8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
      GGCACGAGAC TTCCCAGGT CAGACGTTCT ACTCCAACC TTCGCTGCTT

8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
      ACTCGAGGTG TCCAGTGCCC GGTAAATCGTA AACGTCCACC AGCGCTTTCC

8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
      AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC

8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTTC GGGCTAGGTG
      CATTCGCCCA GAACAAGGGT CGCCAGGGTA GGTTCGAAGC GCCGATCCAG

8401 TCGCGCGGCA GTCCTAGAG GCTCATCTCC GCCGAAGTTC ATGACCAGCA
      AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT

8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
      ACTTCCCGTG CTCGACGAAG GGTTCGCGG GGTAGGTTCA TATCCAGAGA

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*Figure 26I*

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT  
 CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCAGT AACTACACCA  
  
 8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTA  
 CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT  
  
 8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG  
 TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC  
  
 8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT  
 CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCTTA AACTCGGGGA  
  
 8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTGCGCTGC TTGTCCTTGA  
 GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAAC  
  
 8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG  
 GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC  
  
 8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA  
 GCTCGGGTTT CAGGTCTACA GCGCGCGGCC GCCAGCCTCG AACTACTGTT  
  
 8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG  
 GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC  
  
 8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGCGCGG  
 AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCAGCG  
  
 9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT  
 CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA  
  
 9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC  
 GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG  
  
 9101 GCGGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG  
 CCGCCCGCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCG  
  
 9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG  
 ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCCTG GCGGGCCCTC  
  
 9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG  
 TCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCTCG ACCACGACGC  
  
 9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GCGGTTGAT CTCCTGAATC  
 GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAACTA GAGGACTTAG  
  
 9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCC GTGAGCTTGA ACCTGAAAGA  
 ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT  
  
 9351 GAGTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGCC TGGCGCAAAA  
 CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT  
  
 9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC  
 AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J



9501 GCGGCGGAGG TCGTTGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA  
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG  
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA  
GCCCCGCGCT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG  
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTGG  
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC  
AACTATAGGG GGTTCGCGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GCGGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT  
CCGCTTCAAC TTTTGGACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG  
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC  
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC  
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG  
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG  
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CGCCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC  
AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA  
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA  
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAAGGCTGA  
TTTGAGAGC TCTTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG  
CGTGGCACC GCGGCCGTCG CCCGCCGCCA GCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GCGGATGGT  
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG  
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC  
 10501 TCTTGCATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTC  
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG  
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT  
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAA CCGGCATCCA  
 10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCCT CATCGGCTGA  
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT  
 10651 AGCAGGGGCTA GGTGCGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC  
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG  
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CGGTGGTATG  
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC  
 10751 CGCCCGTGTT GATGGTGTA GTGCAGTTGG CCATAACGGA CCAGTTAACC  
 GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCC TGGTCAATTGC  
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC  
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG  
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC  
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG  
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG  
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCCAC  
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA  
 CCGCCCCGAG GCCCCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT  
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG  
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCGCGCGCG  
 11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC  
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG  
 11101 ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA  
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CCGGTTAGCA ACTGCGAGAT  
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG  
 CTGGCACGTT TTCTCTCGG ACATTCGCC GTGAGAAGGC ACCAGACCAC  
 11201 GATAAATTCT CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA  
 CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT  
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA  
 AGGCCGGCAG GCGGCACTAG GTACGCCAAT GGCGGGCGCA CAGCTTGGGT  
 11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG  
 CCACACGCTG CAGTCTGTTG CCCCCTCACG AGGAAAACCG AAGGAAGGTC

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC  
TTCGCCAATC CGACCTTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT  
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGA CTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG  
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCCGCTTG CAAATTCTC CGGAAACAGG GACGAGCCCC TTTTGTGCTT  
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCAGATG CATCCGGTGC TCGGCGAGAT GCGCCCCCTT CCTCAGCAGC  
AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCG

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCTTC CCCTCTCTCT  
CCGTCTCTGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA  
TGCGCGAGTC CTCCCCGTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG  
AATGCTTGGG GGCGCCGCGG CCCGGGCGGT GATGGACCTG AACCTCTCTC

11801 GCGAGGGCCT GGCGCGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG  
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTCC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT  
CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT  
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG  
AGGTGCGTCC GCGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG  
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCGG ACCTGGTAAC CGCATAAGAG CAGACGGTGA  
GCGTGTGCAC CGCCGGCGGC TGGACCATG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT  
TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT  
CACCGCGCGC TCCTCCACCG ATATCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT  
TTCGCGCGAC CTCGTTTGG GTTTATCGTT CGGCGAGTAC CGCGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTAG GGATGCGCTG  
AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG  
GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCGC  
ACCGGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCCATG GACAAGGAGG TAAAGATCGA  
TTCTATATGG TATGGGGAAT GCAAGGGTAT CTGTTCTCTC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC  
CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG  
ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT  
GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTCCCGGGA

12651 GGCTGGCAGC GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG  
CCGACCGTGC CCGTCGCCGC TATCTCTCCG GCTCAGGATG AAATGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG  
CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG  
CGGCC'TGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT  
GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG  
TGATTGCCCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC

12901 CGGTGCGGGC GGCCTGCAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC  
GCCACGCCCG CCGCGACGTC TCGGTCGGCA GGCCGGAATT GAGGTGCCTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC  
CTGACCGCGG TCCAGTACCT GCGGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG  
ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCAGAGAA GGTGCTGGCG  
TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCG ACGAGGCCGG  
TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA  
GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTCGCCGT

13201 ACGTGCAAGC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG  
TGCACGTCTG GTTGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG  
TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGACCGC GCCCTGTCC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA  
TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTT TCCAGACCAG  
GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTC'TGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAA'ACTTGC  
ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTT'TGAACG

13501 AGGGGCTGTG GGGGGTGCGG GCTCCACAG GCGACCGCGC GACCGTGTCT  
TCCCCGACAC CCCCCACGCC CGAGGGTGTG CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT  
TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA  
GTGCC'TGTC CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGT'CAGGCGC ATGTGGACGA GCATACTTTC  
GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGT'CA GCGCGCGCTG GGGCAGGAGG ACACGGGCAG  
GTCTCTAAT GTTACAGTC GCGCGCGGAC CCCGTCTCC TGTGCCCGTC

13751 CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC  
GGACCTCCGT TGGGATTGTA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG  
GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCAGCGT  
GTGCTCTCGC ACTCGGAATT G'GACTACCG CTGCCCCATT GCGGGTCGCA

13901 GCGCGTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA  
CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCATCG CGCGGCCGCC  
TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTAC CAATGCCATC TTGAACCCGC ACTGGCTACC  
CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTGGA GGTGCCCGAG GGTAACGATG  
CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101 GATTCCCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG  
CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA  
TGGGACGATC TCAACGTTGT CCGCTCGTC CGTCTCCGCC GCGACGCTTT

Figure 260

14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC  
GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA  
TCGTGAGCGT GGTGGGCGGG CCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTC  
GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG  
GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG  
ATGCGCGTCC TCGTGTCCTT GCACGGTCCG GCGCGGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG  
AGTTTCCGTG CTGGCAGTCG CCCAGACCA CACCCTCCTG CTACTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTGGGAG GGAGTGGCAA CCCGTTTGCG  
GTCTGCTGTC GTCGCAGGAC CTAAACCCTC CCTCACCGTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA  
GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTTCTT  
ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCCTT TAGTATGCGG CCGCGGCGGA TGTATGAGGA AGGTCTCTCT  
CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG  
GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCACCGCC GCCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC  
AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC  
ACGCCGGATG GCCCCCTCT TTGTCTAGG CAATGAGACT CAACCGTGGG

14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT  
GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA  
CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001 TTCAAAACAA TGACTACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT  
AAGTTTTGTT ACTGATGTCT GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTTGACGACC GGTGCGACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC  
GAACTGCTGG CCAGCGTGAC CCCGCCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGGCG  
GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCGCG

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA  
CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT  
ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG  
CTGGTATCTG GAATACTTGT TCGCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC  
CGTCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG  
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT  
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCCTA

15451 GCGGGGTGGA CTTCACCAC AGCCGCTGA GCAACTTGTT GGGCATCCGC  
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA  
TTCGCCGTTG GGAAGGTCTT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAC ATTCCCGCAC TGTGGATGT GGACGCCTAC CAGGCGAGCT  
CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GGCGGGGGTG GCGCAGGCGG CAGCAACAGC  
ACTTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGCC GTCGTTGTGC

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA  
TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA  
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC  
GTGCCGACT CCTCTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACCAGTGAT  
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA  
GTTTGGGGAC TGTCTCCTGT CGTTCTTTCG GTCAATGTTG GATTATTCTG

15901 ATGACAGCAC CTTCAACCCAG TACCGCAGCT GGTACCTTGC ATACAACCTAC  
TACTGTCTGT GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GCGGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACCTCTGA  
CCGCTGGGAG TCTGGCCTTA GCGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC  
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG  
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 A

16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT  
 CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA  
 16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC  
 AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG  
 16251 ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT  
 TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTCGCA  
 16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG  
 TGGCGACGCG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC  
 16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG  
 GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC  
 16401 CCGCGCGTCC TATCGAGCCG CACTTTTTGA GCAAGCATGT CCATCCTTAT  
 GCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCGTACA GGTAGGAATA  
 16451 ATCGCCACAG AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT  
 TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA  
 16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG  
 AACCGCCCGG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC  
 16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC  
 GTGATGGCGC GCGGGACCCC GCGCGTGTTT GCGCCGGCGT GACCCGCGTG  
 16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA  
 GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT  
 16651 CGCCACGCC GCCACAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG  
 GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC  
 16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT  
 CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA  
 16751 AGCACGTCGC CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG  
 TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCCGCC  
 16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGGC GGCCATGCGG  
 GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC  
 16851 GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG  
 CGCGAGCTT CCGACCGCGC CCCATAACAG TGACACGGGG GGTCCAGGTC  
 16901 GCGACGAGCG GCCGCCGAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG  
 CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC  
 16951 GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC  
 CAGCGTCCCC GTTGCACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG  
 17001 GTGCCCCTGC GCACCCGCCC CCCGCGCAAC TAGATTGCAA GAAAAAATA  
 CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26 R



17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG  
GATACAGGTT CGCGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA  
CTCTAGATAC CGGGGGGCTT CTTCTTCTC GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG  
CGATTTTCGCC CAGTTTTTCT TTTTCTTTCT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG  
TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTTCGAC GCGTAAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT  
TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG  
ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG  
ACATGCCGCT GTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA  
AAACGGATGC CTTTCGCCGT ATTCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC  
CCCCTTGGGT TGTGGATCGG ATTTCCGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT  
GGCGGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTCCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA  
CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC  
TCTACAGAAC CTTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG  
CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTTCTG TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA  
CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GGCGGTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCCTTGC CTCAGCGGTG GCGGATGCCG  
CCCCTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CGGTGCAGGC GGTGCTGCG GCGCGTCCA AGACCTCTAC GGAGGTGCAA  
GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCAGCGCC CGCGCCGTTT  
TGCTTGGCA CCTACAAAGC GCAAAGTCGG GGGGCCCGCG GCGCGCAAG

17951 GAGGAAGTAC GCGCGGCCA GCGCGTACT GCGCAATAT GCCCTACATC  
CTCCTTCATG CCGCGCGGT CGCGCGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG  
TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC  
AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCAGC  
CGCTTCTTCC GTCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTCG

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG  
TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA  
GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TCGCGACCAC  
CCCCGTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCCT  
GCCGCCGCCG CCGCGACGCT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT  
GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG  
GGCACC GGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC

18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC  
TTTTTAGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTITGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC  
ATAAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AACTGGCAA GATATCGGCA CCAGCAATAT  
CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTGCTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT  
CTCGCCACCG CGGAAGTCGA CCCCAGCGA CACCTCGCCG TAATTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA  
AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGACCTT GTCGTGCTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT  
CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC  
CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA  
TCCGTCACGT TTTATTCTAA TTGTCATTCT AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCGTGGCGA  
CTCCTCGGAG GTGGCCGCGA CCTCTGTCAC AGAGGTCTCC CCGCACCGCT

Figure 26 T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT  
TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC  
GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG  
CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCCCCACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC  
CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA  
CGGCGGTCCG CAGGCGCTAG CAACCCCGGG CATCGGTAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC  
TTCGTGTGAC TTGTCTGATG ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC  
CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA  
TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC  
ACCGATGGGG AAGCTACTAC GGCCTCACCA GAATGTACGT GTAGAGCCCG

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG  
GTCCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG  
GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG  
GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGEGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT  
AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT  
GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT  
AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCCTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCAA ATCCTTGCGA  
CCGTGACGGA TGTTCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG  
TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAATCAG  
TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 U

19951 TCAATAGGT GTCGAAGGC AAACACCTAA ATATGCCGAT AAAACATTTT  
AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAG

20001 AACCTGAACC TCAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT  
TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTG TCTTTAATTA

20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA  
GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT

20101 CGGTTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG  
GCCAAGTATA CGTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTA-GAAC

20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTTC  
ATTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT  
AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTC

20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT  
CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301 CTTACATGCC CACTATTAAG GAAGGTAAC CACGAGAACT AATGGGCCAA  
GAATGTACGG GTGATAATC CTTCCATTGA GTGCTCTTGA TTACCCGGTT

20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT  
GTTAGATACG GGTGTCCGG ATTAATGTAA CGAAAATCCC TGTAAAAATA

20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC  
ACCAGATTAC ATAATGTTGT CGTGCCCAT TATACCCACAA GACCGCCCGG

20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AACACAGAG  
TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCTGTC TTTGTGTCTC

20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT  
GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA  
AAGATACACC TTAGTCCGAC AACTGTCGAT ACTAGGTCTA CAATCTTAAT

20601 TTGAAATCA TGGAACGTAA GATGAACCTC CAAATTACTG CTTTCCACTG  
AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC

20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAACAGG  
CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG  
AGTCCTTTTA CCTACCCTTT TTCTACGATG TCTTAAAGT CTATTTTAC

20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC  
TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG

20801 CTGTGGAGAA ATTTCTGTGA CTCCAACATA GCGCTGTATT TGCCCGACAA  
GACACCTCTT TAAAGGACAT GAGGTTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 v

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC  
TGCTGATGTA CTTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCAGCGTG GTCCCTTGAC TATATGGACA ACGTCAACCC  
TAATTGGAAC CTCGTGCGAC CAGGGAACTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG  
TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT  
CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA  
CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC  
GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC  
ATTCCCAACT GCCTCGGTCG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT  
TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA  
ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC  
TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTGTCACGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA  
GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT  
CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC  
TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA  
AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG  
ACTGGCGGAC GAATGGGGGT TGCTCAAAC TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCCTG  
CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC  
CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA  
TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT

Figure 26 W

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC  
CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA  
GTGGTACGCG CTTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT  
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCOA AGAAACGCTA

22001 CGCACCCCTT GCGCATCCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC  
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCACGCGC  
TGAGTGTCTG GACCCGGTTT TGAAGAGAT GCGGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCCAC CCTTCTTTAT  
ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG  
CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA  
GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA  
GTTGTATTTC TTCGTTCTGT GTAGTTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGTTTG TGGGCCATAT  
CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351 TTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA  
AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC  
CGAGCGGACG CCGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGATG

22451 ACTGGATGGC CTTTGCTTGG AACCCGCACT CAAAAACATG CTACCTCTTT  
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA  
CTCGGGAAAC CGAAAAGACT GGTGCGTGAG TTCGTCCAAA TGGTCAAAT

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT  
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC  
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC  
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCCG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC  
GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTGGAATAA TGGCCCCATG

Figure 26 X

22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCGCGAG  
GTCCTTGTGCG AGATGTGCGA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGTCAC TTGAAAAACA  
GGTGTACGCG GTCTAATCCT CGCGGTGAAG AAAAACAGTG AACTTTTTGT

22901 TGTA AAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT  
ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAAA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT  
AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG  
AATTTTTAGT TTCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC  
TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC  
TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC  
GTTGCGCAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC  
GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT  
TGATAGTCGC GGCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT  
GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC  
AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG  
AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT  
TATGTGCGGG ACGTATTTTC GGAAC TAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAC TGATTG  
AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTGGAGAT  
CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG  
GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA  
TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26Y

23701 ATCACGTGCT CCTTATTTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC  
TAGTGACGCA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT  
CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG  
GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG  
TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAG

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG  
GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC  
GAAGGTGAAC CAGTCCGTCA TCAAACCTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA  
TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT  
GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCTCTT GCGTCCGCAT ACCACGCGCC  
GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGCGCGG

24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC  
TGACCCAGCA GAAGTAAGTC GCGGCGGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTGCTGAA ACCCACCATT TGTAGCGCCA  
TAGGAATAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG  
GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC  
GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTCG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA  
GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCCTC  
CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGCGGACG GGGACGGGGA  
TAGGCGAAAA AACCCTCGCG GGGCCCTCCG CCGCCGCTGC CCCTGCCCTT

24501 CGACACGTCC TCCATGGTTG GGGGACGTCG CGCCGCACCG CGTCCGCGCT  
GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGTC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC  
GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC  
ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTGGGATTG



24701 CTACCACCTT CCCCCTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT  
GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA

24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT  
TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA

24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG  
TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCCTCTC CGTTTGCTCC

24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA  
TTGTTTACGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT

24901 GACGACGTGC TGTTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA  
CTGCTGCACG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT

24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCTT CGCCATAGCG GATGTCAGCC  
GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG

25001 TTGCCTACGA ACGCCACCTA TTCTCACCGC GCGTACCCCG CAAACGCCAA  
AACGGATGCT TCGGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT

25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGGTATT  
CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACGCA  
ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT

25151 AGATACCCCT ATCCTGCCGT GCCAACCACA GCCGAGCGGA CAAGCAGCTG  
TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC

25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT  
CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA

25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG  
CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACCTCTG AGTGTTGGTG  
GAGACGTTGT CCTTTTGTCT CTTTTACTTT CAGTGAGACC TCACAACCAC

25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA  
CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

25401 GGTCAACCCAC TTTGCCTACC CGGCACCTAA CCTACCCCGG AAGGTCATGA  
CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT

25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG  
CGTGTCAGTA CTCACTCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA  
CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT

25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGAGAG  
GCTCGTCGAT CGCGCGACCG AAGTTTGCAG GCTCGGACGG CTGAACCTCC

Figure 26 AA

25651 TGCATGCAGC GGTTCCTTTC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA  
ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT

25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA  
TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC  
AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAACACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC  
CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT  
CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC  
CCGTCTGCCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAAACTGCT AAAGCAAAC TTGAAGGACC TATGGACGGC  
TTCTTCGACG TCTTTGACGA TTTCGTTTG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGGACATC ATTTTCCCCG  
GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCTGTAG TAAAAGGGGC

26051 AACGCCTGCT TAAAACCCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA  
TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT  
TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCA GTCTTAGAA

26151 GCGCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC  
CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC  
CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG  
TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGCTACTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC  
AGATGACCTC ACGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT  
ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTTGAA  
CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCAACTT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAA TTTGTACCTG  
TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AACATGGAC

26501 AGGACTACCA CGCCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG  
TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AGCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCTTGG  
GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC

26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG  
GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC

26651 GACGGGGGGT TTACTTGGAC CCCAGTCCG GCGAGGAGCT CAACCCAATC  
CTGCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG

26701 CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA  
GGGGGCGGCG GCGTCGGGAT AGTCGTCGTC GCGCCCCGGG AACGAAGGCT

26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG  
CCTACCGTGG GTTTTCTTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC

26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA  
CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT

26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTGG  
CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC

26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CGGTGCGATT CCCCTCGCCG  
TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC

26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC  
CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG

27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA  
AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCCTGT

27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA  
GGTGACCTTG GTCCCGGCCA TTCAGGTTTCG TCGGCGGCGG CAATCGGGTT

27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC  
CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG TGTTCTTGCG

27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCCGC  
GTATCAACGA ACGAACGTTT TGACACCCCC GTTGTAGAGG AAGCGGCGCG

27201 GCTTTCTTCT CTACCATCAC GCGGTGGCCT TCCCCCGTAA CATCCTGCAT  
CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA

27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA  
ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT

27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA  
GTCGTCGCCG GTGTGTCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT

27351 AAGCCCAAGA AATCCACAGC GCGGCGAGCA GCAGGAGGAG GAGCGCTGCG  
TTCGGGTCTT TTAGGTGTCT CCGCCGTCGT CGTCCTCCTC CTCGCGACGC

27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT  
AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCTTAA

27451 TTCCCACTCT GTATGCTATA TTCAACAGA GCAGGGGCCA AGAACAAGAG  
AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTCTC

Figure 26: AC

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC  
 AGTGTTTTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT  
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC  
 AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCGG

27701 GCCAGCACCT GTTGTGAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT  
 CGGTCTGGA CAACAGTCGC GGTAACTACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA  
 TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC  
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG  
 GGCCAGTTG CCTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC  
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC  
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG  
 GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTCGC CCGGGCAGGG TATAACTCAC  
 GCCCGCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC  
 GACTGTTAGT CTCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GGCGCCGGCC  
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC  
 CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAAT CTGCAATTTA TTGAGGAGTT  
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC  
 ACACGGTAGC CAGATGAAAT TGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC  
 GCCTAGTTAA ATAAGGATTG AACTGCGCC ATTTCTGAG CCGCTGCGG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT  
 ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGC'TTGC CCGCGACTCC GGTGAGTTT  
CCAGGTGACA GCGGCGGTGT TCACGAAACG GCGGCTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGGC GCACGGCGTC  
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCCGAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC  
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG  
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT  
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAAACGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT  
GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG  
TAGCGGTAGG ACATTTGCGG TGGCAGAACT GGGCGGGTTC GTTGTGGTTC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG  
GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT  
AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT  
TGAGGTAGTC TTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT  
CGCAGTGCC GGCAGCGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT  
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA

29051 AGAAAACCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT  
TCTTTTGGGA ATCCCATAAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTG TAATTCAGGT TTCTCTAGAA  
CTTGTTAAGT TCGTTGAGAT GCGCGATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTGG GGTATTCTC TGTCTGTGA TTCTCTTTAT TCTTATACTA  
AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA  
TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT  
AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTAATCACC TTGCGTCAGC CCACGGTACC ACCCAAAGG  
TTAGGATCCA AATGAGTGGG AACGAGTCG GGTGCCATGG TGGGTTTTC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTGCGAGC TGAAGCTAAT  
ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTATTATGCT ATTTGGCAGC  
AGCGGTGTTT TTGTTTTAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT  
GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT  
TTTTGAAAT ACATATGAAA AGGTAAATA CTTTACACGC TGTAAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAT  
CATGTACTCG TTTGTCTATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG  
TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA  
CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCACCACT  
CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA  
TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCCAGTCAT TTCCTGCTCA ATACCATTCC  
TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA  
GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC  
CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCAGGTCGT GGACAGGGCG

30001 GGATTTGTTC CAGTCCAAC ACAGCGACCC ACCCTAACAG AGATGACCAA  
CCTAAACAAG GTCAGGTTGA TGTGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC  
GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG  
GGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG  
AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG  
GACGGATTTT GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC  
ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT  
TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30401 TGC GGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT  
 ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA  
 30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG  
 ACGAAATGCC TAAACAGTGG GAGTCCGAGT AGACGTCGGA GTAGTGACAC  
 30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA  
 CAGTAGCGGA AATAGGTCAC GTAAGTACC CAGACACACG CGAAACGTAT  
 30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA  
 AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT  
 30601 GAATTCCTTA ATTATGAAAT TTAGTGTGAC TTTTCTGCTG ATTATTGCA  
 CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT  
 30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA  
 GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT  
 30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG  
 ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC  
 30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTTC  
 GCTAGAAAGG CTTCCGACCA ATATACGTTA GTAGAGACAA TACCACAAGA  
 30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG  
 CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAAACCGACC  
 30851 AACGCAATAG ATGCCATGAA CCACCCAAC TTCCCCGCGC CCGCTATGCT  
 TTGCGTTATC TACGGTACTT GGTGGGTGA AAGGGGCGCG GCGGATACGA  
 30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC  
 AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG  
 30951 GCCCACCTTC TCCCACCCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA  
 CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT  
 31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC  
 CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG  
 31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT  
 TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGCTACTTA  
 31101 CAAGAGCTCC AAGACATGGT TAACTTGAC CAGTGCAAAA GGGGTATCTT  
 GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA  
 31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC  
 AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG  
 31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG  
 TGGCGGAATC GATGTTCAAC GGTGGGTTTC CAGTCTTTAA CCACCAGTAC  
 31251 GTGGGAGAAA AGCCCATAC CATAACTCAG CACTCGGTAG AAACCGAAGG  
 CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA  
 TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT  
 31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT  
 TATTATTTTC TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA  
 31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC  
 ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG  
 31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC  
 GAGGACCGAC GTTTGAAAGA GGTGTAGAT TTACCTTACA GTCAAAGGAG  
 31551 CTGTTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC  
 GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG  
 31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG  
 CGCGTTCTGG CAGACTTCTA TGGAGTTGG GGCACATAGG TATACTGTGC  
 31651 GAAACCGGTC CTCCAACGTG GCCTTTTCTT ACTCCTCCCT TTGTATCCCC  
 CTTTGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG  
 31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG  
 GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC  
 31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC  
 TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTAA CCCGTTGCCG  
 31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT  
 GAGAGAGACC TGCTCCGGCC GTTGGAAATGG AGGGTTTTAC ATTGGTGACA  
 31851 GAGCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG  
 CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC  
 31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT  
 GTGGGGAGTG TCAATGGAGT CTTCCGGATT GACACCGACG GCGGCGTGGA  
 31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC  
 GATTACCAGC GCGGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG  
 32001 CGTGCACGAC TCCAAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT  
 GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCA  
 32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT  
 GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGCTA  
 32101 AGCAGTACCC TTACTATCAC TGCCTCACC CCTCTAACTA CTGCCACTGG  
 TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC  
 32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAC  
 ATCGAACCCG TAACTGAAC TTCTCGGGTA AATATGTGTT TTACCTTTTG  
 32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT  
 ATCCTGATTT CATGCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH



32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC  
TTGATTTCAA TGACCTCGGA ACCCAAACT AAGTGTTCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA  
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTGTG TCGCGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT  
GAACTACAAT CAATAGGCAA ACTACGAGT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTAA TAAACTCAGC CCACAACCTG GATATTAACT  
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT  
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551 GAGGTAAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT  
CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA  
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA  
TGTGTTTAGG GGAGTTTTGT TTTTAACCGG TACCGGATCT TAAACTAAGT

32701 AACAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC  
TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG

32751 AGGTGCCATT ACAGTAGGAA AAAAAATAA TGATAAGCTA ACTTTGTGGA  
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT  
GGTGTTGGTC AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAACTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT  
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC  
AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CCTTGTCAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC  
TTTCACGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC  
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG  
ACTTCCGTGT CGGATATGTT TCGGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA  
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCATTGTA ACAGTCAGTT

33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT  
CAATGAATT TGCCTCTGTT TTGATTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTCATG GGACTGGTCT GGCCACAAC TACATTAATGA AATATTTGCC  
GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAATTACT TTATAAACGG

33301 ACATCCTCTT AACTTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG  
TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAAC

33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT  
ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC  
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT  
CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC  
GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT  
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTGCG GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA  
AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT  
CGAGTGAATT CAAGTACAGC GACAGGTGCA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT  
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA  
CCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC  
CGCGCGCTTA TTTGACGACG CGGGCGGCGA GGCAGGACGT CTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCCCGA GCATAAGGCG  
TACCGTCACC AGAGGAGTCG CTAATAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCTT GATCTCACTT AAATCAGCAC  
GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG  
TCATTGACGT CGTGTGCTGG TGTATAACA AGTTTATAGG TGTCACGTTT

34001 GCGCTGTATC CAAAGCTCAT GGGGGGGACC ACAGAACCCA CGTGCCCATC  
CGCGACATAG GTTTCGAGTA CCGCCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG  
TATGGTGTTT GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC  
TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG  
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC  
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT  
CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC  
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG

34401 ATTCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA  
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC  
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC  
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTTGG TCGTAGTGTG  
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCTGAAG CAAAACCAGG  
TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC  
ACGCCCACAC TGTGTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC  
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCGCGGG

34751 CCTGGCTTCG GGTCTATGT AAACCTCTTC ATGCGCCGCT GCCCTGATAA  
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCTGTT  
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT  
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG  
AAAAAATAAG GTTTTCTAAT AGGTTTTGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GCGGTGGTCA AACTCTACAG CCAAAGAACA  
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTCCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGACAAT GGCTTCCAAA AGGCAAAACGG  
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC  
GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA  
GGTGGAGAG TTATATAGAG ATTCTGTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA  
AACATTTTAA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA  
TAGTACTAAC GTTTTAAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC  
TCGCCTTGTA ATGTGTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCC

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC  
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC  
GCGGTCTTGT GTACTGTTTT CTGGGTGTG ACTAATACTG TGCGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG  
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA  
GCTATATTTT ACGTTCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC  
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTTC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC  
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTAAACATT  
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA  
TCTTCGGACA GAATGTTGTC CTTTTGTG GGAATATTTC TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA  
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTATGTCC GGAGTCATAA TGTAAGACTC  
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA  
CCATTTGTGT AGTCCAATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC  
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC  
GGGTATCCTC CATATTGTTT TAATTATCCT CTCPTTTTGT GTATTTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA  
ACTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36101 AAAGAAAACC TATTAACAAA ACACCACTCG ACACGGCACC AGCTCAATCA  
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGCGCCGTGG TCGAGTTAGT  
 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA  
 CAGTGTACACA TTTTTCCTCG GTTCACGTCT CGCTCATATA TATCCTGATT  
 36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCAGC  
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC  
 36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCACAAAC TTCCTCAAAT  
 GCTTGGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTG AAGGAGTTTA  
 36301 CGTCACTTCC GTTTTCCAC GTTACGTAC TTCCCATTTT AAGAAAATA  
 GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT  
 36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAACCTA CGTCACCCGC  
 GTTAAGGGTT GTGTATGTTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG  
 36401 CCCGTTCCTA CGCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA  
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGTG TGAGGTGGGG GAGTAATAGT  
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 36451 TATTGGCTTC AATCCAAAAA AAGGTATATT ATTGATGATG TTAATTAAGA  
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACACTAC AATTAATTCT  
 36501 ATTCCGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC  
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG  
 36551 TCGCTTCCGG CGGCATCGGG ATGCCCCGCT TGCAGGCCAT GCTGTCCAGG  
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGTA CGACAGGTCC  
 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG  
 GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC  
 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC  
 CTTGGCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG  
 36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG  
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGG  
 36751 ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT CCCTCGTGCG  
 TGTCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC  
 36801 CTCTCCTGTT CCGACCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG  
 36851 CTTCCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT  
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA  
 36901 TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT  
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

37001 CCGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT  
GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGCC  
TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA  
TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT

37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG  
TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCTGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA  
TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT  
AGTTTTTCTT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT  
CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTCG  
GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TG TAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA  
ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA  
TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG  
GGTCGGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTGCGGGG AAGCTAGAGT AAGTAGTTCTG  
GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT  
GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

37751 GTCACGCTCG TCGTTTGGA TGGCTTCATT CAGCTCCGGT TCCCAACGAT  
CAGTGGGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC  
GTTCCGCTCA ATGTACTAGG GGTACAACA CGTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCGC TGTTATCACT  
AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 AN

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG  
CTACGAAAAG AACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC  
ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT  
GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG  
GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG  
ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA  
GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT

38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT  
ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA  
ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCGAA  
TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401 AAGTGCCACC TGACGTCTAA GAAACCATT TATCATGAC ATTAACCTAT  
TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA  
TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

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38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)  
AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG  
GTAGTAGTTA TTATATGGAA TAAAACCTAA CTCGGTTAT ACTATTACTC

51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG  
CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA  
ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG  
CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG  
CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGCG CCATTTTCGC GGGAAACTG AATAAGAGGA  
GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA  
TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT  
CCCCGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG  
GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT  
CGCCGCGGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTGACATT GATTATTGAC  
ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA  
ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG  
ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT TACGTCAATA ATGACGTATG TTCCCATAGT  
GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT  
TTGCGGTTAT CCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGGCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC  
TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA  
GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTGAT

Figure 27A



901 TCGCTATTAC CATGGTGATG CGGTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCCGCT  
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG  
CCAGTGCGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA  
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG  
GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTEGCGG CGGTGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC  
GGCTGACGCG GACCGACCTC CGGGTCTCTC TGCTCTCTCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA  
CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCAC TTCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT  
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC  
GGGTCTTCTC CGTCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCC

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGCA TCAGGTTCCT  
ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG  
GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC  
ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG

Figure 27B

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT  
GAGGTTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG  
TGTTCCTGAC GATTTCGGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCTC CCCCCTGCCT TCCTTGACCC TGAAGGTGC  
GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCCTG GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC  
GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG  
ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CCTGTCGTTT

2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC  
CCCCCTCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT  
ATACCGGCTA GCGCGCGGCG ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGT ATCTGTTTTG  
CCCTTTCTTA TATATTCCAC CCCAGAATA CATCAAAACA TAGACAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG  
GTCGTCGGCG GCGGCGGTAC TCGTGGTTGA GCAAACCTACC TTCGTAACAC

2301 AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TCGTCAGAA  
TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACCTCTA  
ACACTACCCG AGGTGCTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC  
GATGGAACCTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTAGCCGC TGCAGCCACC GCGCGCGGGA TTGTGACTGA  
AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG  
GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC  
GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTAACTT AAGAAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT  
GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA  
AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT  
TTTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

Figure 27C

2751 TATTTAGGGG TTTTGCGCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC  
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGACTCTGGA  
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC  
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTGG TAGATGATCC AGTCGTAGCA  
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG  
CCTCGCGACC CGCACCACGG ATTTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT  
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTtaggTT  
CCCACGTATG CACCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101 GGCTATGTTC CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA  
CCGATACAAG GGTCCGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTATG TAGCTTAGAA  
GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC  
CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCTG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG  
GTACGTAAAGC AGGTATTACT ACCGTTACCC GGGTGCCCCG CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTC CAGGATGAGA  
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCTTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG  
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA  
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCCACGC TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG  
AAAGGGTGCG AAACCTCAAGT CTACCCCCCT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG  
TACTTCTTTT GCCAAAGGCC CCATCCCCTC TAGTCGACCC TTCTTTCGT

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCCG TAAATCACAC  
CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTATCC  
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTT  
GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

Figure 27D

3701 CCTGACCAAA TCCGCCAGAA GGCGCTCGCC GCCCAGCGAT AGCAGTTCTT  
GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG  
CGTTCCTTCG TTCAAAAAG TTGCCAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTGTAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC  
GAAAACTCGC AAAGTGGTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG  
GACGAGATGC CGTAGAGCTA GGTCTGTATG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG  
CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCTCTG TCAGCGTAGT CTGGGTCACG  
AGTACAGAAA GGTGCCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT  
CACTTCCCCA CGCGAGGCCG GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCTGTCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA  
CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GGTAGCATTT GACCATGGTG TCATAGTCCA GCCCCTCCGC GCGGTGGCCC  
CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG  
AACC CGCGCT CGAACGGGAA CCTCCTCCGC GGCCTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT  
TGAAAACCTC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTG CACGAGCCAG  
TCCGTAGGCG CGGCGTCCGG GCGCTCTGCC AGAGCGTAAG GTGCTCGGTC

4301 GTGAGCTCTG GCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT  
CACTCGAGAC CGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA  
CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCG  
GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC

4451 AGCGGTGTTC CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC  
TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC  
TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTCAAC CTCCCCATCG

4551 GGTCTGTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG  
CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCCACAC TTCTGTGTAC

4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC  
AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT  
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCACT  
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTAC CTGCCCCGCG GTGATGCCTT  
AAGGTTTTTG CTCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA  
ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT  
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTCGCGATC GGCGCGCTCC TTGGCCGCGA  
CCTCGCTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGGCGCT

5001 TGTTTAGCTG CACGTATTCTG CGCGCAACGC ACCGCCATTC GGGAAAGACG  
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTCTGTC

5051 GTGGTGCGCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGTCAG  
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG  
CCTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT  
AGGTCTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCG  
TCGACGAGA GCAGGCCCCC CAGACGAGG TGCCATTCTT GGGGCCCCGTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT  
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTT AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA  
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCTT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC  
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC  
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA  
AAGGTGGCGC CTACGACCGC GCGTGCATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA  
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGACGCT  
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC  
CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT  
CAGATCCCCG GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTCGCGGTCT  
CAGGGAAAAA AAAGGTGTCT AGCGCCAACCT CCTGTTTGAG AAGCGCCAGA

5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC  
AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA  
ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC  
GCCCATCGCG CATA CGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT  
CSTTTCCACA GGGACTGGTA CTGAACTCC ATGACCATAA ACTTCAGTCA

6001 GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG  
CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC  
TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA  
CGCGCTCCGT ATTTCAACGC AACTACGCC TTCCCAGGGC CGTGGAGCCT

6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTGTA  
TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201 TGTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG  
ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACCTAC

6251 GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG  
CTTLCGTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA  
GGGCACGAGA CTTTCCCGG TCAGACGTTT TACTCCCAAC CTTGCTGCT

6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG  
TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401 GTCCTAAACT GGCGACCTAT GGCCATTTT TCTGGGGTGA TGCAGTAGAA  
CAGGATTTGA CCGCTGGATA CCGGTAAAA AGACCCCACT ACGTCATCTT

6451 GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT  
CCATTCGCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACCT CATGACCAGC  
GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTGC

Figure 27G

6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG  
 ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC  
 6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG  
 CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACC GA TAACTACACC  
 6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA  
 ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT  
 6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA  
 TTTTGACACG GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT  
 6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC  
 CCAACTGGAC TGCTGGCGCG GTTCTCTCG TCTACCCCTT AACTCGGGG  
 6851 TCGCCTGGCG GGTTCGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG  
 AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC  
 6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC  
 TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGCGGCG  
 6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTGCGAG CTTGATGACA  
 CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT  
 7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG  
 TGTCGCGCT CTACCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC  
 7051 GTCAGGCGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC  
 CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG  
 7101 GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGGC  
 CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCACCAA CCACCGCCGC  
 7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GCGCGACTA CGGTACCGCG  
 AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC  
 7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCTT GGATGATGCA TCTAAAAGCG  
 GCCGCCCCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC  
 7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA  
 CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCTT GGGCGGCCCT  
 7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC  
 CTCCTCCGTC CCCGTGCAGC CGCGGCGCGC GCGGCTCTC GACCACGACG  
 7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTTGA TCTCCTGAAT  
 CGCGCATCCA ACGACCGCTT CGCTGCTGC GCCGCCAACT AGAGGACTTA  
 7401 CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG  
 GACCGCGGAG ACGCACTTCT GCTGCCCGGG CCACTCGAAC TTGGACTTTC  
 7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAAA  
 TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCCG GACCGCGTTT

Figure 27H

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG  
GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCCA GAAGGCGTTG  
ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCCGCATC  
TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA  
CGCCCGCGCG TACTGGTGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG  
TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTG  
CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA  
CAACTATAGG GGGTTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC  
GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GGCGACAGTG TCGCGCACCT CGCGCTCAAA  
AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT  
CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTTCCGGA

8051 CCCCTTCTTC TTCTTCTGGC GCGGGTGGGG GAGGGGGGAC ACGGCGGCGA  
GGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCTCCCTG TGCCGCCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG  
GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGGCGG GCCGTCTCTG CGGGGGCGCA  
CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCT

8201 GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG  
CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCAACC GCCCCCGAC

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT  
GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG  
TCCATGAGGC GCGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG  
TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCGTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA  
TCGTGGCACC GCGCGCGTC GCGCGCGCC AGCCCCAACA AAGACCGCCT

Figure 27I



8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG  
 AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC  
 8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA  
 AGCCGGTACG GGGTCCGAAG CAAAACTGTA GCCGCGTCCA GAAACATCAT  
 8601 GTCTTGCA TG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTTGTC  
 CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG  
 8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG  
 GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC  
 8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG  
 ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC  
 8751 AAGCAGGGCT AGGTCCGGCA CAACGCGCTC GGCTAATATG GCCTGCTGCA  
 TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT  
 8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT  
 GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA  
 8851 GCGCCCGTGT TGATGGTGTA AGTGCAAGTT GCCATAACGG ACCAGTTAAC  
 CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG  
 8901 GSTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG  
 CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC  
 8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT  
 GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA  
 9001 CCCACCAAAA ACTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT  
 GGGTGGTTTT TCACGCCGCC GCCGACGCC ATCTCCCCGG TCGCATCCCA  
 9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT  
 CCGCCCCGA GGCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA  
 9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC  
 TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG  
 9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC  
 CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG  
 9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT  
 GTACCAGCCC TCGGAGACCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA  
 9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT  
 TCTGGCACGT TTTCTCTCG GACATTCGCC CGTGAGAAGG CACCAGACCA  
 9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT  
 CCTATTAAAG CGTCCCATTA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA  
 9351 ATCCGGCCGT CCGCGTGAT CCATGCGGTT ACCGCCCCGG TGTCGAACCC  
 TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9451 GGC GCG GCG GCT CTGCTGCGCT AGCTTTT TTTG GCCACTGGCC GCGCGCAGCG  
CCGCGCCCGCC GACGACGCGA TCGAAAAAAC CCGTGACCGG CCGCGCTCGC

9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCTGTAG  
ATTGCCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GTTCGAGTC  
GGCCTCCCAA TAAAAGGTTC CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601 TCGGACCGGC CCGACTGCGG CGAACGGGGG TTTGCCCTCCC CGTCATGCAA  
AGCCTGCGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT  
CTGGGGCGAA CGTTTAAGGA GGCTTTTGTC CCTGCTCGGG GAAAAAACGA

9701 TTTCCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG  
AAAGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCCT CCCTCCTCC  
GCCGTTCCTG TTCCTGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG  
ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851 ATTACGAACC CCCGCGGCGC CGGGCCCCGGC ACTACCTGGA CTTGGAGGAG  
TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901 GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG  
CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTT

9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACCC  
CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCC AGGAGATGCG GGATCGAAAG  
ACAAAGCGCT GCGCTCCCT CTCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT  
AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACC GGGATT AGTCCCGCGC  
CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GCGCGCCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG  
CGCGTGTGCA CCGCCGGCGG CTGGACCATT GGCGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAAC TTCA AAAAGCTTT AACACCACG TGC GTACGCT  
TTGGTCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG  
ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG  
ATTGCGCGCA CCTCGTTTGT GGT TATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA  
CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT

10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG  
AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCC  
CACC GGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC

10551 CAAGATATAC CATACCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG  
GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCTC CATTTCTAGC

10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC  
TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG

10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG  
GACCCGCAAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC  
CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCGCGG

10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG  
ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801 GGCGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG  
CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC

10851 GGGCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTGCGCG  
CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCGC

10901 GCGTGAGGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG  
CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG  
ATGATTGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC

11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA  
CGCCACGCCC GCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC  
GCTGACCGCG GTCCAGTACC TGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG  
GACTGCGCAA GGCCGTCGTC GCGTCCGGT TGGCCGAGAG GCGTTAAGAC

11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC  
CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TGCGTGCTCT TCCACGACCG

11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG  
CTAGCATTTG CGCGACCGGC TTTTGTCCCG GTAGCCGGG CTGCTCCGGC

11251 GCCTGGTCTA CGACGCGCTG CTTCAGCGCG TGGCTCGTTA CAACAGCGGC  
CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTGCGCG

Figure 27L

11351 GGC GCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG  
CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC

11401 CACTAAACGC CTTCCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG  
GTGATTGCG GAAGGACTCA TGTGTCGGGC GGTGACACGG CGCCCTGTCT

11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGACTGAGAC  
CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG

11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA  
TGGCGTTTCA CTCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT

11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG  
CATCTGTTCC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTGAAC

11601 CAGGGGCTGT GGGGGGTGCG GGCTCCCACA GGCACCGCG CGACCGTGTC  
GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG

11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT  
ATCGAACGAC TCGGGGTGA GCGCGGACAA CGACGACGAT TATCGCGGGA

11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACTAGG TCACTTGCTG  
AGTGCCCTGTC ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC

11751 AACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT  
TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA

11801 CCAGGAGATT ACAAGTGTC ACGCGCGCT GGGGCAGGAG GACACGGGCA  
GGTCTCTAA GTTCACAGT CCGCGCGCA CCGCTCCTC CTGTGCCCCG

11851 GCCTGGAGGC AACCTAAAC TACCTGCTGA CCAACCGCG GCAGAAGATC  
CGGACCTCCG TTGGGATTG ATGGACGACT GGTGGCCGC CGTCTTCTAG

11901 CCCTCGTTGC ACAGTTTAA CAGCGAGGAG GAGCGCATTT TGCGCTACGT  
GGGAGCAACG GTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA

11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCAGCG  
CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TCGGGGTCGC

12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA  
ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATACGGAGT

12051 AACCGGCCGT TTATCAACCG CTAATGGAC TACTTGATC GCGCGGCCG  
TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGC

12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC  
GCACTTGGGG CTCATAAAGT GGTACGGTA GAACCTGGGC GTGACCGATG

12151 CGCCCCCTGG TTTCTACACC GGGGGATTG AGGTGCCCCA GGGTAACGAT  
GCGGGGAC AAAGATGTGG CCCCTAAGC TCCACGGGT CCCATTGCTA

12201 GGATTCTCT GGGACGACAT AGACGACAGC GTGTTTCCC CGCAACCGCA  
CCTAAGGAGA CCCTGCTGTA TCTGCTGTC CACAAAAGG GCGTTGGCGT

Figure 27 M

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC  
 TCCTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTCCA AGCTTGATAG GGTCTCTTAC  
 GCGCCAGTC TACGATCATC GGGTAAAGGT TCGAATATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCTGCT GGGCGAGGAG GAGTACCTAA  
 GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATT  
 TGTGAGCGA CGACGTCGGC GTCGCGCTTT TTTGGACGG AGGCCGTAAA

12501 CCCAACAAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC  
 GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC  
 CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGACG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG  
 CAGTTTCCGT GCTGGCAGTC GCGCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTGGA GGGAGTGGCA ACCCGTTTGC  
 CGTCTGCTGT CGTCGCAGGA CCTAAACCCT CCCTCACCCT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAAAAAAA AAAAAGCATG  
 CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTTCT  
 TACGTTTTAT TTTTGTAGTG GTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC  
 ACATAAGGGG AATCATACGC GCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG  
 AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTACCCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC  
 CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC  
 GACGCCGGAT GGCCCCCTC TTTGTCGTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG  
 GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTC AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC  
 ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA  
 TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA  
 AGAACTGCTG GCCAGCGTGA CCCCGCCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA  
 GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT  
 13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCCG GGGCAACTAC TCCGAGACCA  
 TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT  
 13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG  
 ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTCAC  
 13401 GGCAGACAGA ACGGGGTTCT GGAAGCGAC ATCGGGGTAA AGTTTGACAC  
 CCGTCTGTCT TGCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACGTG  
 13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG  
 GCGGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC  
 13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA  
 CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAATAAAA CGACGGTCCT  
 13551 TGCGGGGTGG ACTTCACCCA CAGCCGCGCT AGCAACTTGT TGGGCATCCG  
 ACGCCCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC  
 13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG  
 GTTCGCCGTT GGGAAAGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC  
 13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCGTA CCAGGCGAGC  
 TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG  
 13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG  
 AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC  
 13751 CAGTGGCAGC GCGCGGAAG AGAACTCAA CGCGGCAGCC GCGGCAATGC  
 GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTACG  
 13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC  
 TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG  
 13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC  
 TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG  
 13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA  
 GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT  
 13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC  
 AGTTTGGGGA CTGTCTCCTG TCGTCTTTG CGTCAATGTT GGATTATTCG  
 14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACTACTA  
 TTACTGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT  
 14051 CGGCGACCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACCTCTG  
 GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC  
 14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTCTGTTGCC AGACATGATG  
 TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC  
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG  
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCAC  
AAGTTAGCGA AAGGGCTCTT GGTCTAAAC CGCGCGGGCG GTCGGGGTG

14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC  
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGCTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC  
ATGGCGACGC GTTGTCTGAG CCTCCTCAGG TCGTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC  
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA  
CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCCAG CAATAACACA GGCTGGGGCC TGCGCTTCCC AAGCAAGATG  
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG  
AAACCGCCCC GGTCTTCGCG GAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA  
CGTGATGGCG CGCGGGACCC CGCGCGTGT TCGCGCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC  
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCACGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT  
TGCGGGTGCG GCGGTGGTCA CAGGTGTAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCCCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG  
CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG  
ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TCGCGCGCGC

14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG  
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA  
CCGGCGAGCT TCCGACCGGC GCCATAACA GTGACACGGG GGGTCCAGGT

15001 GCGCAGGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG  
CCGCTGCTCG CCGCGGGCGT CGTCGGCGCC GGTAAATCAG ATACTGAGTC

15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG  
CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGGACGC

Figure 27P

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA  
TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC  
CGATACAGGT TCGCGTTTTA GTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA  
CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAACCTGAC  
TCGATTTTCG CCAGTTTTTC TTTTCTTTC TACTACTACT ACTTGAACCT

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG  
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC

15401 GAAAGGTCTGA CGCGTAAAC GTGTTTTCG ACCCGGCACC ACCGTAGTCT  
CTTTCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCCG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG  
AATCGGGGCC ACTCGCGAGG TGGCGTGGA TGTTCGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA  
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTGCTCG CGGAGCCCCCT

15551 GTTGCCTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG  
CAAACGGATG CCTTTCGCCG TATTCTGTA CGACCGCAAC GGCGACCTCG

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG  
TCCCCTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCCTAAAGC GCGAGTCTGG  
GGGCGCAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCG CGCTCAGACC

15701 TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG  
ACTGAACCGT GGGTGGCACG TCGACTACCA TGGGTTCGCG GTCGCTGACC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCAGGGTC  
TTCTACAGAA CCTTTTTCAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGA CTGGGCG TGCAGACCGT  
GCGCACGCCG GTTAGTTCTG CCACCGCGGC CCTGACCCGC ACGTCTGGCA

15851 GGACGTTTCTG ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG  
CCTGCAAGTC TATGGGTGAT GGTTCATCGTG GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGGTTG CCTCAGCGGT GGCGGATGCC  
TCCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCCTACGG

15951 GCGGTGCAGG CGGTCGCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA  
CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGGCGC CCGCGCCGTT  
TTGCCTGGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GGCGCGGCAA

Figure 27A



16051 CGAGGAAGTA CGGCGCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT  
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG  
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC  
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT  
CAGCGGCAGC GGTCGGGCAC GACCGGGGCT AAAGGCACGC GTCCACCCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG  
GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT  
GTAGCAAATT TTCGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGa

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG  
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA  
TCCCCGTACC GGCCGGTGCC GGACTGCCCC CCGTACGCAG CACGCGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTGCGAT GCGCGGCGGT ATCCTGCCCC  
GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA GCGCGCCCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA  
AGGAATAAGG TGA CTAGCGG CGCCGCTAAC GCGGGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAAAAACAA GTTGCATGTG  
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGTG CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA  
CTTTTATGTT TTATTTTCA GACCTGAGAG TGCGAGCGAA CCAGGACATT

16651 CTATTTTGTG GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCC GCGACA  
GATAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA  
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT  
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTAA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC  
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG  
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGa CCTGGCCAAAC  
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT  
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC  
TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCATAAAG CAAGGCCTGC CCACCACCCG  
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGG

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA  
AGGGTAGCGC GGGTACCGAT GGCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA  
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCCACCG CCGTTGTTGT AACCCGTCTT AGCCGCGCGT CCCTGCGCCG  
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGG

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC  
CGGCGGTCG CCAGGCCTA GCAACGCCG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC  
TTTCGTGTGA CTTGTCTAG CACCCAGACC CCCACGTTAG GGAATTGCGC

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC  
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG  
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGGG CGAAAGGTT

17501 ATGGGTACCC CTTGATGAT GCCGCACTGG TCTTACATGC ACATCTCGGG  
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCG GCTGGTGCAG TTTGCCCGG  
GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGT  
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG  
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT  
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC  
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC  
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG  
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAT TAAACCTAGA AGAAGAGGAC  
TTACCCTACT TCGACGATGA CGAGAATTT ATTTGGATCT TCTTCTCCTG

Figure 275

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAACTCA  
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTGTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA  
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTCGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT  
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAA

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA  
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT  
AGTACGTCGA CCCTCTCAGG ATTTTCTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTTATA TGCAAAACCC ACAATGAAA ATGGAGGGCA AGGCATTCTT  
TGCCAAGTAT ACGTTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTT  
CATTTCTGTTG TTTTACCTTT CGATCTTTCA GTTACCTTT ACGTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG  
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT  
ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA  
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA  
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC  
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTTGTAGAT TTGCAAGACA GAAACACAGA  
GTTCTGATCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT  
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT  
AAAGATACAC CTTAGTCCGA CAACTGTCTG TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAACCTG AGATGAACTT CCAAATTACT GCTTCCACT  
TAACTTTTAG TACCTTGAAT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAACAG  
CCCTCCACAC TAATTATGTC TCTGAGAAATG GTTCCATTTT GGATTTTGTG

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAAT  
CAGTCCTTTT ACCTACCTTT TTTCTACGAT GTCTTAAAAG TCTATTTTAA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA  
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTAGTTAG ATTTACGGTT

Figure 27T

18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC  
TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA  
ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT

19051 CATTAACTTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC  
GTAATTGGAA CCTCGTGCGA CCAGGGAACT GATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG  
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCCTC AGAAGTTCTT  
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA  
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTAAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC  
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC  
GATTCCCAAC TGCCTCGGTC GTAATTCAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC  
GTGGAAGAAG GGGTACCGGG TGTTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC  
AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT  
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCGCGG CTGGGCCTTC ACGCGCCTTA  
GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCTATCA CTGGGCTCGG GCTACGACCC TTATTACACC  
TCTGATTCCT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC  
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CTTTGACTC TTCTGTCAGC TGGCCTGGCA  
GAAATTCCTC CACCGGTAAT GGAAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC  
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTAAATTCGC GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCCT  
CCCCTCCCAA TGTTGCAACG GGTACATTG TACTGGTTTC TGACCAAGGA

19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC  
CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC  
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGG TGATACTAAA TACAAGGACT ACCAACAGGT  
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC  
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT  
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA  
TATCCGTTCT GCGGTCAACT GTCGTAATGG GTCTTTTCA AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG  
AGCGTGGGAA ACCCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCACGCG  
GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TCGGTTGAG GCGGGTGGC

20201 CTAGACATGA CTTTGTAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA  
GATCTGTACT GAAAACCTCA CCTAGGGTAC CTGCTCGGGT GGGGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG  
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGGCGC

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CCTTCTCGGC CGGCAACGCC  
CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGGCG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC  
TGTTGTATTT CTCGTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGGTT GTGGGCCATA  
TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA  
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA  
TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT  
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTG  
ACTCGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCAGCCGC  
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC  
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC  
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGCTCGCAA  
GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA  
GGTCTTGTG GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTCA CTGAAAAAC  
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA  
TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG  
AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGG

21101 TTTAAAAATC AAAGGGGTTT TCCCGCGCAT CGCTATGCGC CACTGGCAGG  
AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTAGTGCTC CACTTAACT CAGGCACAAC  
CTGTGCAACG CTATGACCAC AAATCAGGAG GTGAATTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA  
GTAGGCGCCG TCGAGCCACT TCAAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GCGCGCGATA TCTTGAAGTC GCAGTTGGGG  
GGTTGCGCAA ATCGTCCAGC CCGCGGTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCATAC ACAGGGTGC AGCACTGGAA  
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTGCGAGA  
GTGATAGTCG CGGCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC  
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCAGGCT TTGAGTTGCA  
AAACCATCGA CGGAAGGTT TTTCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCCGTC TGGGCGTTAG  
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC  
CTATGTCGCG GACGTATTTT CGGAAGTAGA CGAATTTTCG GTGGAATCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT  
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651 GGCCGGACAG GCCGCGTCGT GCACGAGCA CCTTGCGTCG GTGTTGGAGA  
CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTCCGGCC CACCGGTTCT TCACGATCTT GGCCTTGCTA  
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT  
 TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA  
 21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC  
 GCGGAAGCTA GAGTCGCGTC GCCACGTCCG TGTTCGCGCT CGGGCACCCG  
 21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG  
 AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TCGCGACGTC  
 21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT  
 CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA  
 22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCCATAC GGCCGCCAGA  
 CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT  
 22051 GCTTCCACTT GGTCAAGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC  
 CGAAGGTGAA CCAGTCCGTC ATCAAAC TTCGCGGAAAT CTAGCAATAG  
 22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC  
 GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGGAAAGAGG  
 22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT  
 TGCGTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA  
 22201 TCCGCTTCGC TGGGCTCTTC CTCTTCCTCT TGCGTCCGCA TACCACGCGC  
 AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCAGC  
 22251 CACTGGGTCTG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC  
 GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG  
 22301 CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCACCAT TTGTAGCGCC  
 GTACGAAC TAATCGTGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG  
 22351 ACATCTTCTC TTTCTTCCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG  
 TGTAAGAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC  
 22401 GCGCTCGGGC TTGGGAGAAG GGCGCTTCTT TTTCTTCTTG GCGCAATGG  
 CGCGAGCCCG AACCTCTTC CCGCGAAGAA AAAGAAGAAC CCGCGTTACC  
 22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC  
 GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG  
 22501 AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT  
 TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCTGAGCT ATGCGGCGGA  
 22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG  
 GTAGGCGAAA AAACCCCGC GGGCCCTCC GCGCGCGTG CCCCTGCCCC  
 22601 ACGACACGTC CTCCATGGTT GGGGGACGTC GCGCCGCACC GCGTCCGCGC  
 TGCTGTGCAG GAGGTACCAA CCCCCTGCAG CGCGGCGTGG CGCAGGCGCG  
 22651 TCGGGGGTGG TTTGCGGCTG CTCCTCTTCC CGACTGGCCA TTTCTTCTC  
 AGCCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27 X

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG  
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCG CTTGAGGAGG AGGAAAGTGAT  
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG  
ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG  
ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTCGCTCT CCGTTTGCTC

22951 GAACAAGTCG GCGGGGGGA CGAAAGGCAT GCGGACTACC TAGATGTGGG  
CTTGTTACAG CCGCCCCCT GCTTTCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG  
TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC  
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTCACC GCGTACCCC CCAAACGCCA  
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTCGCGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT  
TCTTTTGCCG TGTACGCTCG GGTGCGCGC GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTT CCAAACTGC  
AACGGCACGG TCTCCACGAA CCGTGGATAG TGTAGAAAAA GGTTTGACG

23251 AAGATACCCC TATCTGCGG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT  
TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTCTGTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG  
CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC  
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT  
CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAATCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG  
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCACCCA CTTTGCCCTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG  
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG  
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG  
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y



23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA  
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG  
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG  
TTTGTAACGT GATGTGGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTT

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA  
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG  
GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC  
GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT  
ACCGTCTGCC GGTACCCGCA AACCCTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG  
GTTCTCGAC GTCTTTGACG ATTTCTGTTT GAACTTCCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC  
GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAAAACCTT GCAACAGGGT CTGCCAGACT TCACCAGTCA  
CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACTTTAT CCTAGAGCGC TCAGGAATCT  
TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAGTAC  
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAACACGG GTAATTCATG

24301 CGCGAATGCC CTCGCGCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC  
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG  
GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC  
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTTCGAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT  
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA  
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAAT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT  
TTGAGTGAGG CCCCACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

24601 GAGGACTACC ACGCCACGA GATTAGGTTT TACGAAGACC AATCCCGCCC  
CTCCTGATGG TCGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG  
CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCC GTGTAAGAAC

24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG  
CGGTAAACGT TCGGTAGTTG TTTGGGCGGG TTCTCAAAGA CGATGCTTTC

24751 GGACGGGGGG TTTACTTGGA CCCCCAGTCC GCGGAGGAGC TCAACCCAAT  
CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA

24801 CCCCCCGCG CCGCAGCCCT ATCAGCAGCA GCCCGGGGCC CTTGCTTCCC  
GGGGGCGGC GCGCTCGGA TAGTCGTCGT CCGCGCCCGG GAACGAAGGG

24851 AGGATGGCAC CAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA  
TCCTACCGTG GGTTTTCTT CGACGTCGAC GCGGCGGGTG GGTGCCTGCT

24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGGAC GAGGAGGAGG  
CCTCCTTATG ACCCTGTCAG TCCGTCTCCT CCAAACCTG CTCTCCTCC

24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC  
TCCTGTACTA CTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTGCGAT TCCCTCGCC  
CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051 GGCGCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC  
CCGCGGGGTC TTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101 CTCAGGCGCC GCCGGCACTG CCCGTTGCGC GACCCAACCG TAGATGGGAC  
GAGTCCGCGG CCGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCTG

25151 ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA  
TGGTGACCTT GGTCCCGGCC ATTCAGGTTT GTCCGGCGCG GCAATCGGGT

25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAAGC  
TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTTCTTGC

25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCGC  
GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301 CGCTTTCTTC TCTACCATCA CCGCGTGGCC TTCCCCGTA ACATCCTGCA  
GCGAAAGAAG AGATGCTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT

25351 TTAATACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA  
AATGATGGCA GTAGAGATGT CCGGTATGAC GTGGCCGCCG TCGCCGTCGT

25401 ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC  
TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG

25451 AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC  
TTTCGGGTTC TTTAGGTGTC GCCGCCGTCG TCGTCTCCTT CCTCGCGACG

25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT  
CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCTTAA

Figure 27 AA

25551 TTTCCCACTC TGTATGCTAT ATTTCACAG AGCAGGGGCC AAGAACAAGA  
AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTCT

25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT  
CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTGCGCGCA CGCTGGAAGA CGCGGAGGCT  
TAGTGTTTTT GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT  
GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG  
AAGAGTTTAA ATTGCGGCTT TTGATGCAGT AGAGGTCGCC GGTGTGGGCG

25801 CGCCAGCACC TGTGTGCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC  
CGGGTCGTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGC GGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCCA  
ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT  
TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCAGAA ACCGAATTCT CCTGGAACAG  
GGGCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC  
CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC  
GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAACTCAGG GGCGCAGCTT  
GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA  
CGCCCCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTTCAGCT CAACGACGAG TCGGTGAGCT  
GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC  
GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAACCTCTG AGACCTCGTC  
GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT  
GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCTTCT CGGGACCTCC CGGCCACTAT  
AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG  
GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTCCTGA GCCGCCTGCC

Figure 27 AB

26501 CTACGACTGA ATGTTAAGTG GAGAGGCAGA GCAACTGCGC CTGAAACACC  
GATGCTGACT TACAATTACAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT  
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCCG GGATCATATC GAGGGCCCCG CGCACGGCGT  
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA  
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCCTACT  
GGGTGCGGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCTTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA  
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAATATA CTGGGGCTCC  
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG  
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGGTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA  
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC  
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG  
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCCTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT  
ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT  
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA  
ATCTTTTGGG AATCCCATAA TCCGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA  
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT  
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCTG CTGTGTGCAC ATTTGCATTT  
TTGCCAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA  
TAACAGTCGA AAAATTTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG  
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTC

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTGCGAG CTGAAGCTAA  
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA  
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG  
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA  
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA  
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGTT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA  
ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT  
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTA CTCTATA TTAATACAA AAGCAGACGC AGCTTTATTG  
CCAGACATGG GATGAGATAT AATTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC  
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT  
ATTGACGAAA TGAGCGACGA ACGTTTGTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATTC  
TTAATCTTAT CCTAAATTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA  
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTGCGGA TGTTGGAAC

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG  
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCTG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA  
GCCTAAACAA GGTCAGGTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA  
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG  
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT  
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT  
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAAATCCAT AGATTGGACG GACTGAAACA  
CACGATGTGG GTTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28451 TTTTATATTA CTGACCCCTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG  
AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT  
GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA

28551 TTGCTTTACG GATTTGTAC C CTCACGCTC ATCTGCAGCC TCATCACTGT  
AACGAAATGC CTAAACAGTG GGAGTCCGAG TAGACGTCGG AGTAGTGACA

28601 GGTCAATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT  
CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT  
TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA

28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTCTGCT GATTATTGCG  
TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC  
TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAA  
TACGTCTAAG TGAGCATATA CTTATAAGG TTCAACGATG TTACTTTTTT

28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC  
CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901 TCCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG  
ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAAACGAC

28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCGCG CCCGCTATGC  
CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT  
AAGGTGACGT TGTTCACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051 CGCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG  
GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG  
TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC

29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA  
GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201 TCAAGAGCTC CAAGACATGG TTAACCTGCA CCAGTGCAAA AGGGGTATCT  
AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCCATAGA

29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA  
AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCATT ATGGTGGCCT

29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT  
GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTA ACCACCAGTA

Figure 27AE

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCCTTATT  
CGACGTAAGT GAGTGGAAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA  
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAATCAG TTAGCAAATT TCTGTCCAGT  
TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT  
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT  
GGAGGACCGA CGTTTGAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCTCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG  
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC  
GCGCGTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACG TGCCTTTTCT TACTCCTCCC TTGTATCCC  
CCTTTGGCCA GGAGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCTGGGG TACTCTCTTT GCGCCTATCC  
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG  
CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAAT GTAACCACTG  
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTAA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT  
ACTCGGTGG AGAGTTTTT TGGTTCAGTT TGTATTGGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC  
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GGCGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA  
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGCACGA CTCCAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG  
GGCACGTGCT GAGGTTTGAA TCGTAACGST GGGTTCCTGG GGAGTGTAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA  
AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG  
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA  
CATCGAACCC GTAACGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC  
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAATAAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA  
TTTGATTTC AATGACCTCGG AACCCAAAAC TAAGTGTTC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT  
GAATTACATC GTCTCTCTGA TTCCTAACTA AGAGTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC  
TGAATAACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAACTCAG CCCACAACCTT GGATATTAAC  
ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTACAGCT TCAAACAATT CCAAAAAGCT  
ATGTTGTTTC CGGAAATGAA CAAATGTGCA AGTTTGTAA GGTTTTTCGA

30651 TGAGGTAAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA  
ACTCCAATTG GATTCTGTGAC GGTCCCCCAA CTACAACTG CGATGTGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA  
ATCGGTAATT ACGTCTCTA CCCGAACCTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTC  
TTGTGTTTAG GGGAGTTTG TTTTAAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA  
TTGTTCCTGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAATGTCTGT

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG  
GTCCACGGTA ATGTCATCCT TTGTTTTAT TACTATTCGA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC  
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTACGTC TCTTCTACG

30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAATA CTTGCTACAG  
ATTTGAGTGA AACCAGAAAT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTCAAGTTT GGCTGTTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT  
AAAGTCAAAA CCGACAATT CCGTCAAACC GAGGTATAG ACCTTGTCAG

31051 CAAAGTGCTC ATCTTATTAT AAGATTGAC GAAAATGGAG TGCTACTAAA  
GTTTCACGAG TAGAATAATA TTCTAACTG CTTTACCTC ACGATGATTT

31101 CAATTCTTTC CTGGACCCAG AATATTGGA CTTTAGAAAT GGAGATCTTA  
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTG GATTATGCC TAACCTATCA  
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCACTCA  
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG



31251 AGTTTACTTA AACGGAGACA AAACATAACC TGTAACACTA ACCATTACAC  
TCAAATGAAT TTGCCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG  
ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTCACG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTGTC  
AGTAAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTT CATACTTGC CCAAGAATAA AGAATCGTTT  
GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTCAA TTGCAGAAAA TTTCAAGTCA  
CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTC AGTAGTATAG CCCACCACC ACATAGCTTA TACAGATCAC  
AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCTAG TATTCAACCT GCCACCTCCC  
GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCC GGCTGG CCTTAAAAAG  
AGSGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG  
GTAGTATAGT ACCCATGTG TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC  
AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCC

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG  
TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA  
AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC  
ACCCCCATCT CAGTATTAGC ACGTAGTCTT ATCCCGCCAC CACGACGTGC

31901 AGCGCGCGAA TAAACTGCTG CCGCGCGCGC TCCGTCCTGC AGGAATACAA  
TCGCGCGCTT ATTTGACGAC GCGCGCGCGC AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC  
GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCCT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA  
CGGAACAGGA GGCCCGTGTC GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA  
GTCATTGACG TCGTGTCTGT GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGCCAT  
CCGCGACATA GGTTCGAGT ACCGCCCCTG GTGCTTTGGG TGCACCGSTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG  
GTATGGTGTG CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC  
 GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG  
 32301 AGCTGGCCAA AACCTGCCCCG CCGGCTATAC ACTGCAGGGA ACCGGGACTG  
 TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC  
 32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT  
 CTGTGTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA  
 32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC  
 GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG  
 32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGAACAACC  
 AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTGG  
 32501 CATTCTTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA  
 GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT  
 32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT  
 TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA  
 32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC  
 GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCCTCC ATCTGCTAGG  
 32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTG GTCGTAGTGT  
 GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAAC CAGCATCACA  
 32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAACCAG  
 GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTGGTC  
 32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG  
 CACGCCCCGA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC  
 32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC  
 GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG  
 32851 CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA  
 GGGACCGAAG CCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT  
 32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCGTT  
 TGTAGGTGGT GCGCTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA  
 32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT  
 GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA  
 33001 TTTTTTTATT CCAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA  
 AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT  
 33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAATCTACA GCCAAAGAAC  
 CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG  
 33101 AGATAATGGC ATTTGTAAGA TGTGACAA TGGCTTCCAA AAGGCAAACG  
 TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC  
GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC  
CGGTGGAAGA GTTATATAGA GATTCTTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG  
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGAAAGTCG AGTTCGTCGC

33351 AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA  
TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTGCGAGGG  
TTCGCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC  
GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCACACA CTGATTATGA CACGCATACT  
GGCGGTCTTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG  
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC  
CGCTATATTT TACGTTCCAC GACGAGTTT TTAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG  
TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTCTCTCA AACATGTCTG  
GAGGCCCTTG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT  
GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTGT TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG  
ATCTTCGGAC AGAATGTTGT CCTTTTGTGTT GGAATATTG GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA  
TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT

33901 AAGCACCACC GACAGCTCCT CGGTCAATGC CGGAGTCATA ATGTAAGACT  
TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG  
GCCATTTGTG TAGTCCAAC TAAAGTAGCC AGTCACGATT TTTCGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC  
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC  
GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG

Figure 27A J

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34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA
      TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT

34201 AAAAGAAAAC CTATTA AAAA AACACCACTC GACACGGCAC CAGCTCAATC
      TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA
      TCAGTGTACG ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT

34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
      TTTTACTGCG ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG

34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCTCAA
      CGCTTGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401 TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATT TTAAGAAACT
      AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA

34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
      TGTAAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGA TGCAGTGGGC

34501 CCCC GTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC
      GGGGCAAGGG TCGGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

                                     PacI
                                     -----
34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
      TATAACCGAA GTTAGGTTTT ATTCCATATA ATAAC TACTA CAATTAATTC

34601 AATTCGGATC TGCACGCGA GGCTGGATGG CCTTCCCAT TATGATTCTT
      TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651 CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG
      GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
      CGTCCATCTA CTGCTGGTAG TCCCTGTGCA AGTCCGGTC GTTTTCCGGT

34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
      CCTTGGCATT TTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG

34801 CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
      GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
      CTGTCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCAGC

34901 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
      CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG

34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
      GGAAGCCCTT CGCACC GCGA AAGAGTATCG AGTGCGACAT CCATAGAGTC

35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG
      AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTGGGGGGGC

```

Figure 27 AK

AAGTCGGGCT GGCACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT  
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC  
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG  
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAACA  
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC  
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTCTGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT  
CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT  
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA  
TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTTCATATAT

35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC  
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCGAC

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC  
CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA  
TTACTATGGC GCTCTGGGTG CGAGTGCCG AGGTCTAAAT AGTCGTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC  
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC  
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG  
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA  
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAG CGGTTAGCTC  
AGTTCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGCCGCA GTGTTATCAC  
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG

Figure 2 AL

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA  
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA  
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT  
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT  
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA  
CATTGGGTGA GCACGTGGGT TGA CTAGATAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA  
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCTT

36351 ATAAGGGCGA CACGGAATG TTGAATACTC ATACTCTTCC TTTTCAATA  
TATTCCTGCT GTGCCTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTG  
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAA CAAATAGGGG TTCGCGCAC ATTTCCCCGA  
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA  
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TCTTCAAGAA TTGGATCCGA  
ATTTTATCC GCATAGTGCT CCGGGAAGC AGAAGTCTT AACCTAGGCT

## PacI

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36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

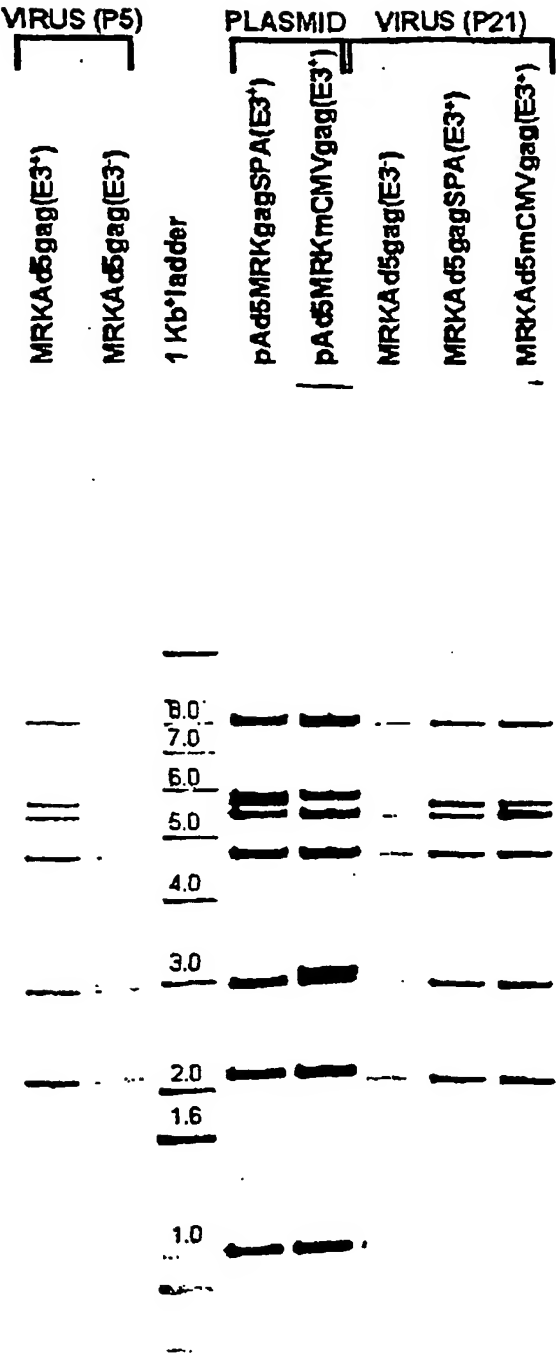


FIGURE 28

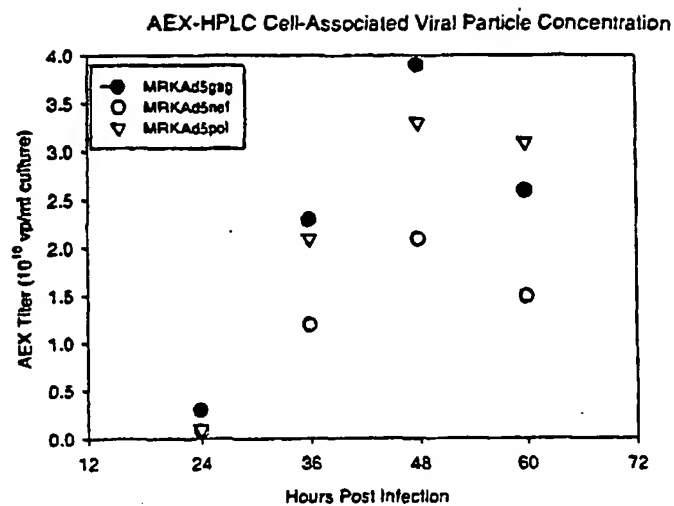


FIGURE 29A

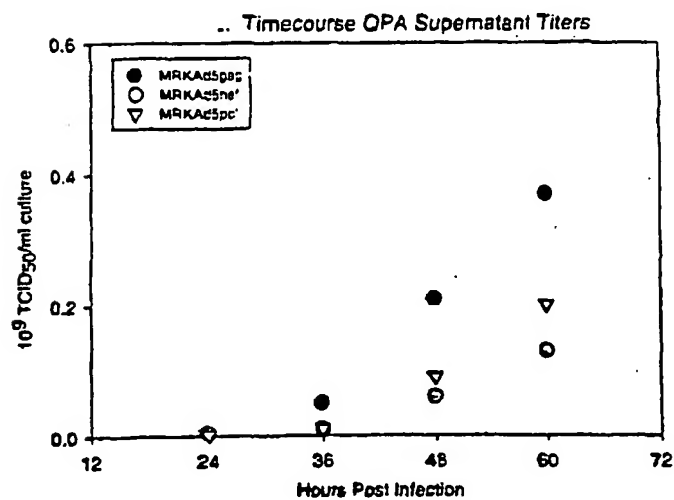


FIGURE 29B



|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga<br>Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly<br>1 5 10 15       | 48  |
| gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg<br>Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg<br>20 25 30        | 96  |
| gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag<br>Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu<br>35 40 45        | 144 |
| ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc<br>Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly<br>50 55 60        | 192 |
| tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt<br>Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys<br>65 70 75 80     | 240 |
| gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag<br>Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys<br>85 90 95        | 288 |
| att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct<br>Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala<br>100 105 110     | 336 |
| gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg<br>Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val<br>115 120 125     | 384 |
| cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc<br>Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr<br>130 135 140     | 432 |
| ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag<br>Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu<br>145 150 155 160 | 480 |
| gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac<br>Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp<br>165 170 175     | 528 |
| ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag<br>Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln<br>180 185 190     | 576 |
| atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg<br>Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu<br>195 200 205     | 624 |
| cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc<br>His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro<br>210 215 220     | 672 |
| agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att<br>Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile<br>225 230 235 240 | 720 |
| ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag<br>Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys<br>245 250 255     | 768 |

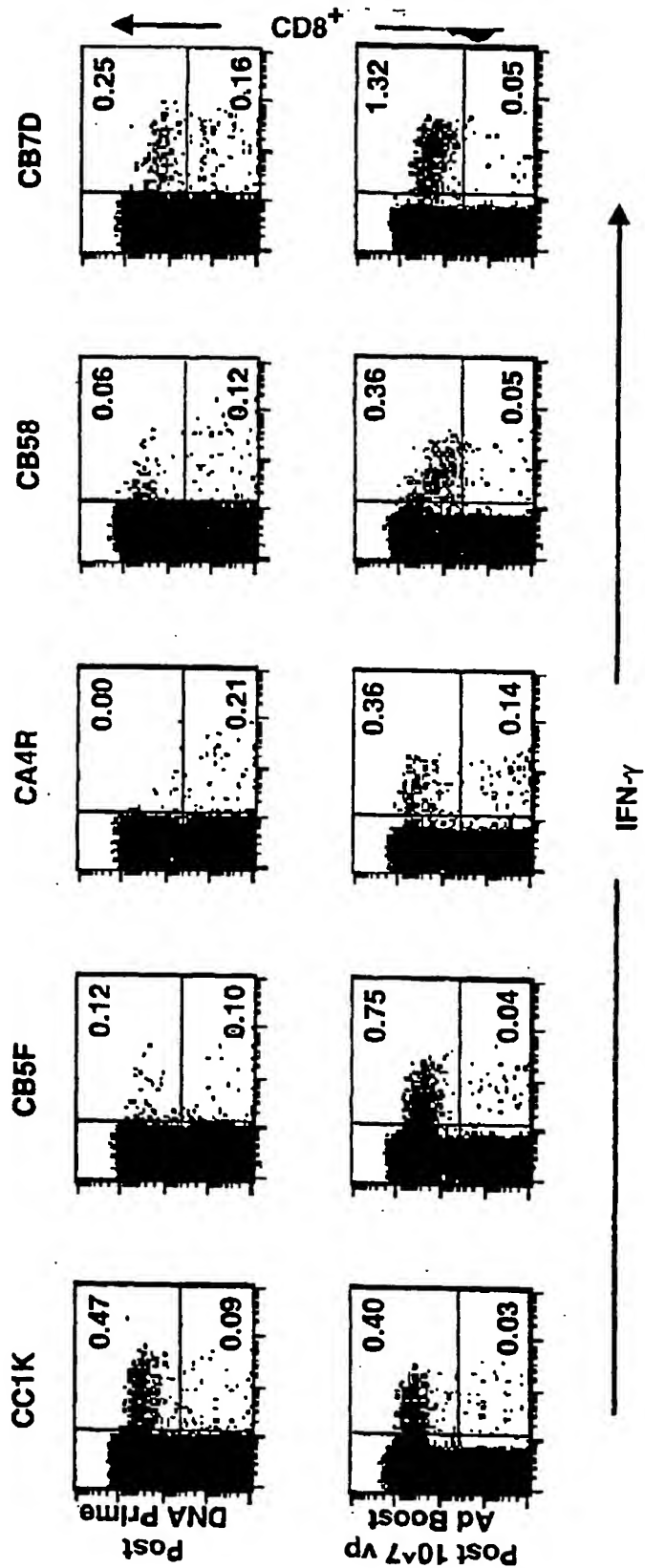
Figure 30A

|                                                                                                                                                          |      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc<br>Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro<br>260 265 270        | 816  |
| acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac<br>Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp<br>275 280 285        | 864  |
| tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag<br>Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln<br>290 295 300        | 912  |
| gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac<br>Glu Val Lys Asn Trp Met Thr Glu Thr Leu Val Gln Asn Ala Asn<br>305 310 315 320        | 960  |
| cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg<br>Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu<br>325 330 335        | 1008 |
| gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag<br>Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys<br>340 345 350        | 1056 |
| gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc<br>Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr<br>355 360 365        | 1104 |
| atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag<br>Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys<br>370 375 380        | 1152 |
| tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc<br>Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala<br>385 390 395 400    | 1200 |
| ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg<br>Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met<br>405 410 415        | 1248 |
| aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc<br>Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro<br>420 425 430        | 1296 |
| tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc<br>Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro<br>435 440 445        | 1344 |
| aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc<br>Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr<br>450 455 460        | 1392 |
| ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc<br>Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala<br>465 470 475 480    | 1440 |
| tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482<br>Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37)<br>485 490 |      |

Figure 30 B

**Figure 31**

**IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs**



# Immunizations

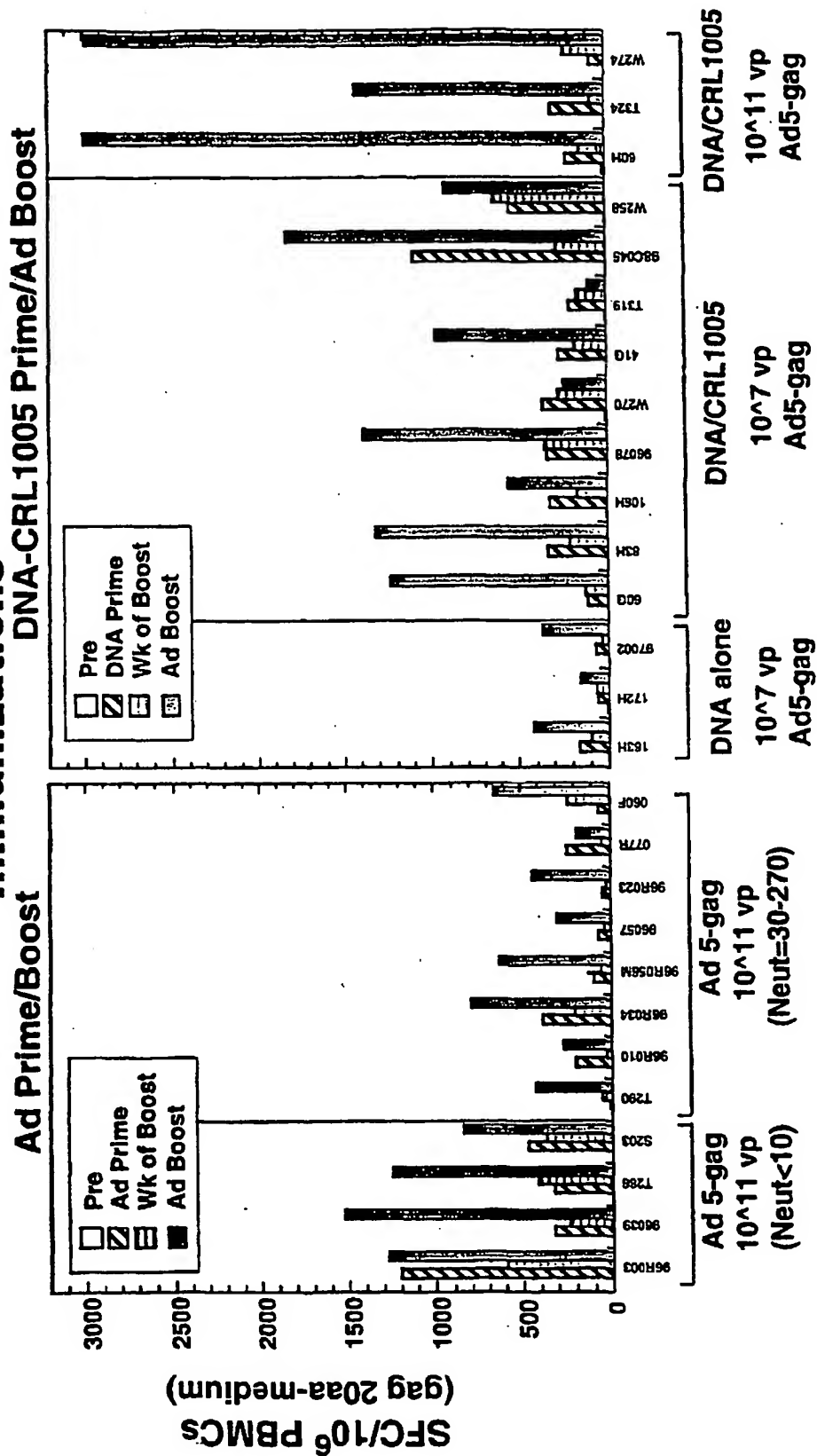


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
 GGCACAGSCA ACTCCAGCCA GGTGTCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
 GAGAAGGCCT TCTCCCTTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA  
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAAGATTG TGAGGATGTA CTCCCCACC  
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT  
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG  
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC  
 ATGCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC  
 CACAAGGGCA GGCCTGGCAA CTTCTCCAG TCCAGGCCTG AGCCACAGC CCCTCCCGAG  
 GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCAGCC AGAAGCAGGA GCCATTGAC  
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCAG  
 ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
 CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC  
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
 GAGCTGAACA AGAGGACCCA GGAATTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
 GGCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCA CTTCTCTGTG  
 CCCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG  
 ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
 AAGAAGCACC AGAAGGAGCC CCCCTTCTTG TGGATGGGCT ATGAGCTGCA CCCCAGACAAG  
 TGGACTGTGC AGCCCATTTG GCTGCCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCCA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTGTGAAC  
ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGGTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAATTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACCTC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA  
SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
 Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
 Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
 Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
 Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
 Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
 Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
 Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
 Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
 Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
 Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
 Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
 Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
 Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
 Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
 Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
 Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
 Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
 Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
 Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
 Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
 Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
 Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
 Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
 Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
 Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
 Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
 Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
 Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
 SEQ ID NO: 39



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category *  | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                              | Relevant to claim No.                                        |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| X<br>—<br>Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.                                                                                                                                          | 1-3, 8-11, 18<br>-----<br>4, 5, 13-17, 29-32, 34,<br>35, 37  |
| X<br>—<br>Y | US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.                                                                                                                                                              | 1-3, 8-11, 18<br>-----<br>4, 5, 13-17, 29-32, 34,<br>35, 37  |
| X,P         | US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.                                                                                                                                                  | 1, 9, 18                                                     |
| X<br>—<br>Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.                                                                                                                                                             | 1-3, 8, 9-11, 18<br>-----<br>4,5,13-17, 29-32, 34,<br>35, 37 |
| Y           | WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18                                             |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;"

document member of the same patent family

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

19 AUG 2002

Name and mailing address of the ISA/US

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                        | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y          | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.                                        | 1, 9, 29-32           |
| Y          | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.                          | 1, 9, 29-32           |
| Y          | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9                  |
| Y          | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.                                                                                                    | 16                    |
| Y          | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.                                                                                                                                     | 1, 9                  |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

| Group | Claims                                       |                                                                                                                                                                                                                                                                                                                                                                               |
|-------|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1     | 1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal. |
| 2     | 6, 7, 36                                     | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).                                                                                                      |
| 3     | 12, 33                                       | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.                                                                                          |
| 4     | 19-23, 38-42                                 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.                                                                                                                                                                                                                           |
| 5     | 24, 27, 28, 43, 46, 47                       | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.                                                                                                                                                                                                                              |
| 6     | 25, 26, 44, 45                               | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                                                                                           |
| 7     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.                                                                           |
| 8     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.                                                                           |
| 9     | 48-51, 53, 54, 56                            | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.                                                                           |
| 10    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.                                                                         |
| 11    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.                                                                         |
| 12    | 52                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.                                                                         |
| 13    | 55                                           | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u>                                                                                                                                                                                                                                                                                |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

|    |                   |                                                                                                                                                                                                                                                                                                         |
|----|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                   | and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.                                                                                                                  |
| 14 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.                  |
| 15 | 55                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.                  |
| 16 | 57-61             | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.                                                                                                                                                     |
| 17 | 62, 65, 66        | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.                                                                                                                                                        |
| 18 | 63, 64            | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                                     |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.    |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.   |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.   |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.   |
| 23 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.  |
| 24 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1. |
| 25 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1. |
| 26 | 71                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1. |
| 27 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.                  |
| 28 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.                 |
| 29 | 74                | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type                                                                                                                |

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|    |             |                                                                                                                                                                                                                                                                                         |
|----|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |             | adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.                                                                                                                                                                                          |
| 30 | 74          | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. |
| 31 | 76-80       | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.                                                                                                                                     |
| 32 | 81, 84, 85  | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.                                                                                                                                              |
| 33 | 82, 83      | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.                                                                                           |
| 34 | 86a         | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.                                                                                                                                                 |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.                                                                                                                                                 |
| 36 | 86c, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .                                                                            |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .                                                                            |
| 38 | 86e, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .                                                                            |
| 39 | 86f, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.                                                                                                                                   |
| 40 | 86g, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.                                                                                                                                                              |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.                                                                                                                                                             |
| 42 | 86i, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.                                                                                                                                                              |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.                                                                                                                                                        |
| 44 | 86k, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.                                                                                                                                                             |
| 46 | 86m, 88     | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 47 | 86n, 88     | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |
| 48 | 86o, 88     | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.                                                                                                                                                      |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter